Chemical restraint of southern elephant seals (*Mirounga leonina*)

by

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Statement of Sources

This thesis contains no material which has been accepted for the award of any other degree or diploma in any tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

Rupert Woods.

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Abstract

This study examined the use of anaesthetic agents in southern elephant seals (*Mirounga leonina*). The aim was to improve their safety especially when used for the routine chemical restraint required for biological investigations. This was to be achieved by more accurately refining drug dose rates, determining appropriate drugs to use and developing techniques for the early recognition and treatment of complications.

Monitoring to detect complications early

A system of monitoring was developed from reflexes and responses used to measure the depth of anaesthesia in terrestrial animals. Heart rate, respiratory rate, rectal temperature, head response, palpebral response, withdrawal response, caudal flipper response, muscle tone, righting response, and capillary refill were used as indicators of anaesthetic depth and the status of the animal's respiratory, cardiovascular, and thermoregulatory systems. It was more difficult to elicit responses when pethidine based drug combinations were used, or with large seals (> 1000 kg), and apnoea made assessment of level of restraint unreliable. However, the responses measured usually followed predictable sequences as level of restraint increased and the system allowed early recognition of complications and comparisons to be made between animals restrained with different drug combinations.

Methods to more accurately estimate animal masses and anaesthetic drug doses

Length-mass relationships were established by linear regression of data gathered from southern elephant seals which had been weighed and measured at Macquarie Island, South Georgia and Heard Island. The relationships differed between locations and with sex and stage of yearly cycle. Although the variability was often large, these relationships offer the best current estimate of mass based on morphometric data and can be used to improve the accuracy of drug dosage.

For certain well defined categories of seals, nominal dosages of ketamine and diazepam were determined which could be administered when an accurate assessment of mass is not possible. Nominal masses, snout-tail lengths, as well as respiratory rates, heart rates and other variables used during monitoring were found for each category of seal. This improved the ability to accurately assess the anaesthetic episode by allowing recognition of abnormal responses and facilitated their early treatment.

Pharmacokinetic studies were carried out in animals at different stages of their yearly cycle. Ketamine concentrations were measured in venous blood after administration of a single intravenous bolus of ketamine to animals which had been sedated with pethidine. The data indicate that apparent volume of distribution, total body clearance and half life may not vary with stage of yearly cycle and that

additional intravenous ketamine dosage is best based on lean body mass. Recommendations were developed for additional drug administration which enable more precise drug dosage, and lessen the problem of inappropriate drug dosage.

Finding the most appropriate anaesthetic drug or combination of drugs

A comparison was made of the responses of groups of seals to the commonly used anaesthetic agents ketamine combined with diazepam, xylazine or midazolam, and tiletamine combined with zolazepam. It was found that all of the drug combinations could be safely used to restrain southern elephant seals. However, for people with little experience with anaesthetics, tiletamine and zolazepam offered some advantages over other combinations with respect to formulation and predictability. However, the results indicated that the safety margin for tiletamine plus zolazepam may not be high and its use should be approached with caution in animals whose mass is not known.

Other anaesthetic drugs which had not previously been administered to southern elephant seals, but had proved useful in other animals, were also examined. These were combinations of midazolam, pethidine, ketamine and thiopentone, and medetomidine combined with ketamine. Pethidine based combinations were very useful and versatile, and were rapidly reversible with naloxone or naltrexone. However, in some cases apnoea was induced after administering ketamine or thiopentone intravenously and the time to maximum sedation was longer than when using cyclohexamine based combinations. Hyperthermia, vomiting and unpredictable responses were associated with the use of medetomidine combined with ketamine and for this reason ketamine combined with xylazine appears preferable.

Treatment of complications

A study was carried out to determine whether antagonists could be used to improve control of the anaesthetic episode. Yohimbine antagonised ketamine combined with xylazine, but antagonism was unnecessary for the cyclohexamine based drug combinations (those containing ketamine or tiletamine) due to the normally rapid recovery from these agents. The use of 4-aminopyridine and sarmazenil to antagonise ketamine combined with diazepam appears to be contraindicated as their administration was associated with prolonged recovery or blindness. Antagonists were not effective in reversing apnoea.

An attempt was made to treat apnoea by firstly determining an effective dose of the respiratory stimulant doxapram in breathing animals and then administering it by a variety of routes to apnoeic animals. Doxapram was not always effective, however endotracheal administration appeared to be of benefit in some cases and is worth trying if facilities to administer positive pressure ventilation are not available. Supportive techniques such as intubation and positive pressure ventilation remain the treatment of choice for apnoea. Pharmacokinetic studies of ketamine in apnoeic and breathing seals

indicated that apnoea affects apparent volume of distribution and total body clearance of this drug during anaesthesia. Recommendations were developed for the prevention and treatment of apnoea.

This study fulfilled its aims of improving anaesthetic safety for southern elephant seals. However, it is still difficult to treat apnoea in large southern elephant seals and techniques for positive pressure ventilation in these animals need to be determined.

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Glossary

Abbreviations used

DI to V	Digit I to V
df	Degrees of freedom
ECG	Electrocardiogram
EEG	Electroencephalogram
FG	French gauge
G	Gauge
h	Hour
X.HCl	Hydrochloride salt of X
IU	International units
L	Litre
mb	Millibar
Saline	0.9% normal saline
PI to V	Phalanx I to V
Pv_{CO2}	Partial pressure in mmHg of carbon dioxide in venous blood taken from the extradural intravertebral vein. Pa_{CO2} (partial pressure of CO_2 in arterial blood expressed in mmHg) gives an indication of ventilatory efficiency and its relation to metabolic rate (West 1985, Cullen 1988).
Pv_{O2}	Partial pressure in mmHg of oxygen in venous blood taken from the extradural intravertebral vein. Pa_{O2} (partial pressure of O_2 in arterial blood expressed in mmHg) gives an indication of lung function (West 1985, Cullen 1988).
Total CO_2	Total carbon dioxide. The sum of the CO_2, carbonic acid and bicarbonate dissolved in plasma. The total CO_2 or CO_2 content derived from an arterial sample is an estimate of the metabolic component of acid-base balance (Cullen 1988).

Terms and definitions

Analeptic — A drug which stimulates respiration (Flecknell 1987).

Anoxia — Literally means without oxygen. Through common usage, it has come to mean inability of tissues to receive or utilize an adequate amount of oxygen. Since a literal anoxia seldom if ever exists, the word hypoxia, indicating a state of decreased oxygen availability or diminished utilization of oxygen by tissues (impaired tissue respiration) has been suggested as being a preferable term (Lumb and Jones 1984 p123).

Antagonist — A drug which inhibits, prevents or reverses the action of another drug.

Anxiolytic — A drug which has an anti-anxiety effect (eg diazepam).

Apnoea — Failure to breath for a period of longer than one min in southern elephant seals (Mitchell and Burton 1991).

Asystole — Lack of cardiac muscle contractions (Flecknell 1987).

Barbiturates — A group of drugs classed as hypnotics. In the central nervous system they cause sedation, hypnosis and medullary depression. They cause a depression in the respiratory centre, often producing apnoea, and have a hypotensive effect on the cardiovascular system due to peripheral vasodilation and a decreased cardiac output (Gales 1989).

Base excess/ base deficit — Seen in alkalosis or acidosis, respectively. Base excess or deficit refer to the quantity of acid or base needed when titrating plasma pH to 7.4 at 37°C while the P_{CO2} is held at 40 mm Hg. Some texts use the term negative base excess instead of base deficit (Cullen 1988). In this volume the term base excess is used and a minus sign used to indicate deficit.

Benzodiazepines — Benzodiazepine derivatives (eg chlordiazepoxide, diazepam, midazolam, zolazepam). Benzodiazepines are used in animals primarily to treat seizures or are given prophylactically to prevent the anticipated seizure activity of other drugs such as ketamine but are also used as muscle relaxants, anxiolytics, and hypnotics (Booth 1982 d, Gleed 1987).

Blood gases — pH, P_{CO2}, P_{O2}, HCO_3^-, total CO_2 and base excess values, usually from an arterial sample, however in this study they refer to venous values from the extradural intravertebral vein. The last 3 values are derived from the pH and P_{CO2}.

Caudal flipper response — (See Table 4.1 and Chapter 4).

Chemical restraint — Includes tranquillization, sedation, immobilization and anaesthesia which represent a continuum of increasing central nervous system depression. Three

broad terms have been used to describe levels of chemical restraint: sedation is used when the animal is conscious and can move its body or a part of its body more than a few cm from the point of touch; immobilization is used when the animal is conscious but unable to move its body or a part of its body more than a few cm from the point of touch; anaesthesia is used when the animal is unconscious and unable to move its body or a part of its body more than a few cm from the point of touch. How these different levels of chemical restraint have been further divided is described in Chapter 4.

Cyanosis — Blue or purple colouring of the skin or visible membranes due to the presence of an increased concentration of reduced haemoglobin in capillary blood, symptomatic of hypoxia (Flecknell 1987).

Cyclohexamines — A common term used to refer to the dissociative anaesthetics (eg ketamine, tiletamine and phencyclidine) which are a group of related chemical compounds that are capable of producing a state of anaesthesia during which the subject has an apparent dissociation from the environment (Short 1987 b).

Cyclohexamine drug combinations — A combination of a cyclohexamine drug with a tranquillizer or sedative, administered intramuscularly to chemically restrain an animal (eg ketamine and diazepam, ketamine and xylazine, tiletamine and zolazepam).

Dyspnoea — Laboured breathing (Lumb and Jones 1984 p101).

Eupnea — Ordinary quiet breathing (Lumb and Jones 1984 p101).

Head response — (See Table 4.1 and Chapter 4).

Hyperthermia — Rectal temperature greater than 38.5°C.

Hypoxia — A state of decreased oxygen availability or diminished oxygen utilization by tissues (Lumb and Jones 1984 p123). (See also anoxia.)

Menace response — The eye preservation or menace response is tested by means of a threatening gesture made towards one eye. The normal response is for the eye to blink and the head to be averted (Eger *et al.* 1988). It assesses the optic nerve and is a test of vision. One eye is usually tested whilst the other is covered, however in this study the opposite eye was not covered.

Narcosis — A state of insensibility or stupor from which it is difficult to arouse the animal (Flecknell 1987).

Narcotic — A group of drugs which has its action in the central nervous system and spinal cord where they alleviate pain and cause some degree of central nervous sytem, cardiovascular and respiratory depression (Gales 1989) and includes such drugs as pethidine, fentanyl, and morphine.

Nociceptive — Describing nerve fibres, endings, or pathways that are concerned with the condition of pain. A nociceptive stimulus is one which stimulates these areas.

Philtrum — Median groove separating the right and left parts of the upper lip.

Prolonged apnoea Apnoea of > 10 min duration.

Prolonged chemical restraint

Periods of chemical restraint where the time to recovery (level 1 of chemical restraint) was > 60 min.

Relapse Used with respect to narcotic antagonist use. The action of some narcotic antagonists is shorter than the narcotic which they antagonise. When the antagonist wears off the animal may therefore become re-narcotised or relapse back into narcosis.

Sedation A mild degree of central depression in which the patient is relaxed and unconcerned by its surroundings (Lumb and Jones 1984). In this study sedation is taken to mean that animals can move forward or swivel around (see chemical restraint).

Suspected hyperthermia

Due to the relatively low levels of chemical restraint used during most parts of this study rectal temperature could not often be measured. Suspected hyperthermia was therefore based upon suspicion of behavioural mechanisms of cooling, specifically when animals were seen to either flick sand or wallow contents over themselves, or move into wallows during or after recovery from chemical restraint. Flicking behaviour, however, may be a non-specific sign of stress not associated with hyperthermia (Laws 1956b), hence the term 'suspected'.

Sympatric Originating in, or occupying, the same geographical area.

Tachypnea High respiratory rate (Lumb and Jones 1984 p101).

Tranquillizers and sedatives

The distinction between tranquillizers and sedatives is a question of semantics and of little value except that increased doses of tranquillizers tend to produce side effects but not loss of consciousness, whereas increased doses of sedatives produces more profound central nervous system depression that resembles anaesthesia (Gleed 1987). Tranquillizers administered to southern elephant seals include the phenothiazine promazine, and sedatives include the benzodiazepines and xylazine (see Tranquillization, and Sedation).

Tranquillization Is a state of behavioural change in which the animal is relaxed and unconcerned by its surroundings (Lumb and Jones 1984 p101) (similar to sedation).

Wallow — A depression in the ground which adult elephant seals often use when they come ashore to moult. Wallow areas are usually established in tussock grassland just behind beaches, and are used by more than one seal. Wallows become a foetid mixture of living seals, seal urine, faeces and moulted skin. When wallows occur on deep peats they may become pools of thick mud (Selkirk *et al.* 1990).

Withdrawal response (see Table 4.1 and Chapter 4).

Nomenclature

Blood vessels: blood vessels are referred to using the nomenclature used to describe the blood vessels of the harp seal *Phoca groenlandica* (St. Pierre 1974).

Drugs are referred to in the text by the nonproprietary names listed in Table 3.2. The counterion has been dropped unless included in the dose rate.

For time course data zero time is when initial administration of anaesthetic drugs was completed (eg "apnoea commenced at 20 min" indicates that 20 min from completion of initial administration of anaesthetic drug apnoea commenced).

Drug dose

Unless otherwise indicated drug dose is presented in mg/kg free drug, and otherwise as the salt. Three different terms are used to describe drug dosage: (1) intended dose or the dose intended to be administered, (2) actual dose or the dose actually administered (determined after weighing the animal) and (3) a nominal dose or the approximate dose found to be suitable for a particular category of animal.

Chapter 1: General Introduction and background to the study

Three families of living pinnipeds, or seals, are recognised: Phocidae (known as "true", "earless" or "phocid" seals), Otariidae (fur seals and sea lions, collectively known as "otariid" seals) and Odobenidae (walrus) (Ling 1983).

Elephant seals are phocid seals. There are two species, southern (*Mirounga leonina*) and northern (*M. angostirostris*). Many aspects of the biology of southern elephant seals have been studied (Selkirk *et al.* 1990). They spend most of their lives at sea in Antarctic and subantarctic waters undergoing long migrations to feeding grounds to replenish fat reserves that are lost when they go ashore on isolated subantarctic islands to breed and moult.

There has been an estimated 50% decrease in the number of Southern elephant seals at Heard Island (53°01'S, 73°23'E) and Macquarie Island (54°30'S, 158°54'E) since 1949 (Burton 1986, Hindell and Burton 1987). Since 1985 the Australian Antarctic Division has been researching this decline. As the animals are large and potentially dangerous, chemical restraint is used to allow weighing, measuring, gastric lavage, blood taking, milk sampling, and deployment and retrieval of automatic data logging devices such as time-depth recorders and satellite tracking devices.

During the austral summer of 1987, 316 southern elephant seals were anaesthetised at Macquarie or Heard Islands with ketamine, ketamine combined with diazepam or xylazine, or tiletamine and zolazepam (Woods *et al.* 1989, Mitchell and Burton 1991). There were 16 fatalities and many problems associated with anaesthesia.

The following problems were found.

- Apnoea, bradycardia, prolonged anaesthesia, shaking, hyperthermia and fatalities were common.
- The response of the animals to anaesthesia was inconsistent.
- There was little control over the anaesthetic episode and no effective way of treating complications associated with anaesthesia. Large animals (≥ 1000 kg) appeared to be a particularly high anaesthetic risk.
- There was little information available on response of elephant seals to anaesthetics. Information was available on response of other seals to anaesthetics (Gales 1989), but prior

to 1987 only 3 studies, representing 44 anaesthetic episodes, had been published on the use of these drugs in adult southern elephant seals (Morgan *et al.* 1978, Ryding 1982, Gales and Burton 1987a).

The following possible solutions were proposed to these problems.

Improve monitoring so that complications could be recognised early

A system of monitoring could be developed by testing reflexes and responses used to measure the depth of anaesthesia in terrestrial animals. Those which are appropriate, and possibly other reflexes and responses, specific to seals, could be used as indicators of anaesthetic depth and the status of the animal's respiratory, cardiovascular, and thermoregulatory systems. A standard system of monitoring might also allow comparison of different drug combinations. (See Chapter 4. Monitoring during chemical restraint.)

Develop methods to more accurately estimate animals' masses and anaesthetic drug doses

More accurate estimates of mass could be made from length-mass relationships for animals of different ages and sexes and at different times of the year. An alternative could be to develop standardised total drug dosages for seals at different stages of their life cycle. Examination of drug kinetics might also aid rational drug dosage. These studies could enable more precise drug dosage, and would lessen the problem of inappropriate drug dosage. (See Chapter 5. Length-mass relationships in southern elephant seals; Chapter 6. Use of ketamine and diazepam; and Chapter 7. Pharmacokinetics of intravenously administered ketamine.)

Find the most appropriate drug or combination of drugs

This could be determined by comparing the responses of groups of seals to commonly used anaesthetic agents. The most appropriate anaesthetics would be those which have the lowest fatality rate, and cause the least respiratory, central, cardiovascular and thermoregulatory depressant effects. Other anaesthetic drugs that had not been administered to southern elephant seals, but had proved useful in other animals or seals, could be examined. (See Chapter 8. A comparison of some cyclohexamine based drug combinations for chemical restraint of southern elephant seals; Chapter 9. Use of midazolam, pethidine, ketamine and thiopentone for chemical restraint of southern elephant seals; and Chapter 10. Medetomidine and ketamine use in southern elephant seals.)

Develop a course of action to treat complications

Control of the anaesthetic episode might be improved by using anaesthetic antagonists. Use of chemical stimulation of respiration or techniques such as intubation and positive pressure ventilation could be used to treat respiratory complications. Neuromuscular blocking agents or skeletal muscle relaxant drugs could be used to treat seizures. (See Chapter 11. Use of the respiratory stimulant doxapram; Chapter 12. Antagonism of some cyclohexamine based drug combinations used for chemical restraint; Chapter 13. Apnoea during chemical restraint; and Appendicies IV and VI, fatalities and complications.)

The study was designed to test the unifying hypothesis that:

the safety to the animals of the anaesthetic episode could be improved by using the most appropriate drugs, more accurately refining drug dose rates and developing techniques for the early recognition and treatment of complications associated with drug administration.

Because this study was to act as a guide to anaesthesia of these animals for people without advanced training in anaesthetics, techniques were needed that could be used safely and be understood by lay people.

A proposal was submitted to The Antarctic Animal Care and Ionising Radiation Usage Ethics Committee (Department of the Arts, Sport, the Environment, Tourism and Territories) to test the unifying hypothesis. Approval was granted but with the exclusion of gaseous anaesthesia as a decision on the ethics of this could not be reached in the time available. The study was also largely restricted to mature pre-moulting females, animals considered to be "low-risk" by the Ethics Committee (Appendix I).

What follows is the result of this work.

Chapter 2: Literature Review

Introduction

Southern elephant seals are large and potentially dangerous. Many aspects of research, husbandry and medicine of these animals require chemical restraint or anaesthesia.

In the field physical restraint is routinely used for pups and weaners. In captivity elephant seals can be conditioned to allow blood sampling (Rogers personal communication) or have been restrained physically by throwing a hoop net over the bigger animals and having several people hold the animal down by straddling it (Gage personal communication). Physical restraint of older or larger animals in the field however can be dangerous, inefficient, have the potential to cause excessive stress to the animal and has only rarely been used (Ross and Saayman 1970; Figure 25-12, Cornell 1978).

Electroimmobilization has not been used with elephant seals. Aversion to electroimmobilization has been reported in cattle and sheep (Pascoe 1986, Pascoe and McDonell 1986, Rushen and Congdon 1986) and it has been suggested that the technique is inhumane when surgery is performed without the addition of local anaesthetic (Trim 1987). The National Health and Medical Research Council *et al.* (1990) proposed that electroimmobilization devices should not be used as an alternative to analgesia or anaesthesia in animals until there was scientifically acceptable evidence that they are effective.

In the field situation where numbers of people available are often limited and with difficulties in transporting capture equipment over rugged terrain or for long distances, chemical restraint is the technique of choice for allowing safe access to these animals.

Many papers have been written on pinniped anaesthesia (Gales 1989), however the majority are largely anecdotal, presenting drug dose levels, numbers of animals anaesthetised and numbers which died; there are few data regarding the normal response to anaesthesia, dosage has largely been determined on a trial and error basis and conclusions drawn are largely unsubstantiated. Hammond and Elsner (1977) present a very good discussion of the problems of phocid anaesthesia and Gales (1989) published a review of the current literature regarding pinniped anaesthesia. Another important publication is Mitchell and Burton (1991) which discusses fatalities associated with chemical restraint of southern elephant seals. The following review presents information largely on southern elephant seal anaesthesia. This information is generally applicable to northern elephant seal anaesthesia. However, those interested in northern elephant seal anaesthesia are specifically referred to Gales (1989) for general comments and a list of drugs and dose levels used in this species. There are no specific studies of any of the drugs in southern elephant seals. The pharmacology of the drugs mentioned in this thesis is described in standard texts of veterinary anaesthesiology and pharmacology such as Booth and McDonald (1982) and Lumb and Jones (1984).

Methods of drug administration

General

Anaesthetic agents have been administered by several routes including intramuscular, intravenous, intrapleurally, intralingually and by mouth using a variety of techniques. There are no reports of gaseous administration.

Intramuscular administration

i. General

This route of drug administration has been the most commonly used for initial drug administration (Table 2.1).

Elephant seals are so lethargic that they can be stalked quite readily and injected at close range (Ling *et al.* 1967). The seals tend to pivot round to face the operator when disturbed, but an assistant standing quietly in front of the seal can usually hold the animal's attention long enough to allow injection. It has been suggested that more care would probably be needed if adults were approached during pupping or breeding seasons (Cline *et al.* 1969).

Though drugs have been administered at various sites (Ling *et al.* 1967), the preferred site for intramuscular injection is the gluteal mass in the dorsal hip area or the lumbar muscles (Parry *et al.* 1981). These areas have readily accessible muscle with a comparatively thin blubber layer (Trillmich and Weisner 1979, Gales 1989).

The advantages of intramuscular administration are that it is easy, safer for personnel than intravenous administration and does not require a detailed knowledge of anatomy. Frequently it is the only option available in the field. There are disadvantages. The delay of onset of effects means that dose cannot be titrated to effect. Injections are often given in haste and intravenous administration and overdosing can occur (Gales 1989). The large volume of drug that is often required to immobilize larger animals also presents a problem. This has been reduced to some degree by using highly concentrated solutions of drugs such as ketamine (Woods *et al.* 1989), increasing the concentration of anaesthetic drugs administered by making them up from powder with the other drugs used in the combination acting as diluent (Baker *et al.* 1988), or using lyophilized drugs such as tiletamine and zolazepam (Baker *et al.* 1990).

Abscessation has been reported after intramuscular drug administration (Gales and Burton 1987 a). One animal was unable to use its hind flippers for approximately 2 weeks. The abscesses healed within 3 weeks, but the animals remained at the moult site for considerably longer than other

Table 2.1. Drugs used to chemically restrain southern elephant seals. Where possible (unless otherwise stated) a mean ± standard deviation is presented for drug dose; where not possible a range is given. Significant figures are those presented by each author. The dose of tiletamine and zolazepam is presented as the dose of combined drug as this was the practice adopted by the authors. n represents the number of animals to which drugs were administered; the number in brackets represents the total number of immobilizations. Multiple dose and additional dose differ in semantics only and are reported as they were in the specified text. (i.m. = intramuscular, i.v. = intravenous, p. o. = by mouth.)

Drug or drug combination	Drug	Route of administration	n	Single dose for induction* (mg/kg)	Multiple dose for induction† (mg/kg)	Total dose‡ (mg/kg)	Useful dose§ (mg/kg)	Additional dose‖ (mg/kg)	Comments	Reference
Phencyclidine with promazine	Phencyclidine	i.m.	5	0.16-0.47			0.4		Variable results	Cline et al. 1969
	Promazine	i.m.		0.16-0.47			0.4			
	Phencyclidine	i.m.	14	0.4						Morgan et al. 1978
	Promazine	i.m.		0.4						
Phencyclidine	Phencyclidine	i.m /p o.	1		540 mg total dose	740 mg total dose		2 doses of 100 mg	Complete immobilisation	Ross and Saayman 1970
	Phencyclidine	i.m.	23	0.28-0.84				phencyclidine i.m.	Violent convulsions, prolonged recovery. Treated with 0.5 mg/kg promazine i.v.	Morgan et al. 1978
Ketamine with xylazine	Ketamine	i.m.	?		8.71±0.25¶ (5.4-12.50)	3.31±0 13 (2.38-3.97) mg/kg/h (at Heard Island) 11.49±0.79 (3.75-18.2) mg/kg/h (at Vestfold Hills)		1.31±0.15 (0-2.50) mg/kg/h ketamine i.v. or i.m.	Good immobilization	Gales and Burton 1987a
	Xylazine	i.m.			0.41±0.01 (0.24-0.85)					
	Ketamine	i.m.	5	3.59-4.91	6.10-6.38					Bester 1988
	Xylazine	i.m.		0.67-1.02	1.13-1.20				Relatively safe and effective	
	Ketamine	i.m.	55 (60)	4.1 ± 0.8 (2.8-6.5)		2.1-11.4		0.9-3.7 ketamine i.m.	Prolonged apnoea, 2 fatalities	Mitchell and Burton 1991
	Xylazine	i.m.		0.2-0.5		0.2-0.5				
	Ketamine	i.m.	181(226)	3.61±1.25					8 fatalities including hyperthermia	Woods et al. 1989
	Xylazine	i.m.		0.55±1.5 to 0.75±0.22						
Ketamine with diazepam	Ketamine	i.m.	?		8.71±0.25¶ (5.4-12.50)	3.31±0.13 (2.38-3.97) mg/kg/h (at Heard Island) 11.49±0.79 (3.75-18.2) mg/kg/h (at Vestfold Hills)		1.31±0 15 (0-2.50) mg/kg/h ketamine i.m. or i.v.	1 fatality, generally reliable results	Gales and Burton 1987a
	Diazepam	i.m.			0.09±0.01 (0 03-1.02)					
	Ketamine	i.m.	26(43)	5.45±1.35 (2.67-9.50)	8.10±1.75 (6.02-11.89)		6		20:1 ratio	Baker et al. 1988
	Diazepam	i.m.					0.03			
	Ketamine	i.m.	42			5.4 (average dose)			50:1 ratio	McCann et al. 1989
	Diazepam	i.m.								
Ketamine		i.m.	5	4.6-13.6	6.06-7.56			1.58-2.93 ketamine i.m.	Good immobilization	Ryding 1982
		i.m.	?	2.0-2.3					Repeated tranquilisation	Ross personal communication
		i.m.	?	2.5					Sedation	Griffiths et al. 1979
		i.m.	6	2.0-6.3						Morgan et al. 1978

*Dose used where induction resulted from a single injection of drug(s).
†Combined dose of drug(s) used where induction resulted from more than 1 injection of drug(s).
‡Total amount of drug administered including single and multiple doses for induction and additional doses for maintenance.
§Dose suggested by the author(s) as being useful or representative.
‖Additional dose administered to induce, maintain or adjust level of chemical restraint. The particular drug administered in brackets.
¶mean ± standard error.

Table 2.1. (Continued). Drugs used to chemically restrain southern elephant seals. Where possible (unless otherwise stated) a mean ± standard deviation is presented for drug dose; where not possible a range is given. Significant figures are those presented by each author. The dose of tiletamine and zolazepam is presented as the dose of combined drug as this was the practice adopted by the authors. n represents the number of animals to which drugs were administered; the number in brackets represents the total number of immobilizations. Multiple dose and additional dose differ in semantics only and are reported as they were in the specified text. (i.m. = intramuscular, i.v. = intravenous, p. o. = by mouth.)

Drug or drug combination	Drug	Route of administration	n	Single dose for induction* (mg/kg)	Multiple dose for induction† (mg/kg)	Total dose‡ (mg/kg)	Useful dose§ (mg/kg)	Additional dose‖ (mg/kg)	Comments	Reference
Suxamethonium		i.m.	31	0.5-2.0					Apnoea common, variable results	Ling and Nicholls 1963
		i.m.	114	0.5-5.5			2.5		5 fatalities, variable results	Ling et al. 1967
		i.m.	75	1.87±0.35					Pups, apnoea at > 3.0 mg/kg	Condy 1980
		i.m.	?	1.3-1.5					Little or no apnoea. Additional physical restraint needed	Griffiths et al. 1979
Suxamethonium with pentobarbitone	Suxamethonium Pentobarbitone	i.m. Intrapleural	?						Third stage anaesthesia prior to euthanasia	Bryden 1971
	Suxamethonium Pentobarbitone	i.m. i.v.	?			20	20		Prior to euthanasia	Griffiths 1980
Xylazine	Xylazine	i.m.	68	1.0-6.3			2.0-2.9 and 3.0-3.9		3 fatalities, hyperthermia, variable results	Vergani 1985
Tiletamine with zolazepam		i.m.	90(195)	0.95±0.22	1.15±0.37		1	Small amounts of tiletamine and zolazepam i.v.	Predictable, 5 cases of apnoea, 1 laryngeal spasm, best immobilizing agent available	Baker et al. 1990
		i.m.	5	2.0-2.4	2.4			0.6-0.8 tiletamine and zolazepam i.m.	Prolonged apnoea, 2 deaths	Mitchell and Burton 1991

*Dose used where induction resulted from a single injection of drug(s).
†Combined dose of drug(s) used where induction resulted from more than 1 injection of drug(s).
‡Total amount of drug administered including single and multiple doses for induction and additional doses for maintenance.
§Dose suggested by the author(s) as being useful or representative.
‖Additional dose administered to induce, maintain or adjust level of chemical restraint. The particular drug administered in brackets.
¶mean ± standard error.

individuals. Hubbard (1969) reported that; because pinnipeds defecate indiscriminately, and the fact that in captivity they will defecate in pool water, the skin is usually quite contaminated. He experienced needle abscessation in pinnipeds more often than in any other species that he had worked with. He found that application of alcohol-saturated cotton to the proposed injection site was ineffective in preventing infection. Hubbard (1969) routinely prepared all animals for injection by hosing them thoroughly, then dousing the entire body with a bucket of antiseptic. The antiseptic was allowed to sit on the skin for ten minutes before an injection was made (Hubbard 1969). Disinfection of the skin prior to drug administration in unrestrained animals is considered impractical under field conditions and is probably unnecessary given the relatively low incidence of injection abscesses reported in the literature.

ii. Techniques

Four basic techniques have been used to administer the initial anaesthetic dose intramuscularly: direct injection with a hand held needle and syringe (Ross and Saayman 1970, Morgan *et al.* 1978, Condy 1980, Ryding 1982, Bester 1988); extension syringes consisting of a long pole with the syringe attached to one end (Ling and Nicholls 1963, Ling *et al.* 1967, Cline *et al.* 1969, Morgan *et al.* 1978, Griffiths *et al.* 1979); remote methods using a long length of plastic tubing with a needle at one end and the syringe containing the drugs at the other (Ryding 1982, Gales and Burton 1987 a, Bester 1988, Woods *et al.* 1989, Mitchell and Burton 1991); and projectile syringes fired from rifles (Vergani 1985) or blowpipes (Baker *et al.* 1988 and 1990).

Direct injection and use of extension syringes are now rarely used other than on weaners. However, Cline *et al.* (1969) believed the extension syringe device to be more workable than projectile syringes for drug administration, both in pack ice and shore situations, except in those few instances where animals could not be approached to within 3 m. Ryding (1982) found that although direct intramuscular injection was satisfactory for females and pups, it was impractical in the case of adult males. The aggressive reaction of a large seal, coupled with the long injection time associated with the large volume of drug needed, made this method of administration impractical. Use of a spring loaded syringe was attempted so that retreat could be effected as soon as the needle had been inserted into the hip region. Invariably the seal reared, violently withdrew its hind quarters and the syringe was displaced. Because of these problems a remote injection technique or administration by projectile are now the commonly used techniques for drug administration.

Ryding (1982) described a remote injection technique which has been the basis for other remote injection techniques. A 90 mm, 18 G needle was connected to a series of 2 lengths of transparent plastic intravenous drip tubing. The tubing was filled with a dose of ketamine and the operator end of the tubing was closed by a clamp to avoid loss until injection commenced. The needle was then

inserted into the caudal pelvic musculature and the drugs injected through the lumen of the tube followed by water or air to flush them through. The play allowed by the tubing provides enough latitude for the dose to be injected in one smooth action should the seal rear and withdraw its hind quarters after placement of the needle (Bester 1988). Subsequent authors modified this technique (Gales and Burton 1987 a, Bester 1988, Woods *et al.* 1989, Mitchell and Burton 1991). Despite its proven usefulness, problems have been described. Gales and Burton (1987 a) found that the large surface area of the tube resulted in the technique becoming unworkable at temperatures less than -15^{o}C because ice blocked the tube (a problem also reported for extension syringes; Cline *et al.* 1969). It was found that a hand warmer alleviated the problem. The positioning of the needle in the dorsal hip area has also been described as important (Cline *et al.* 1969, Gales and Burton 1987 a). If the needle was pressed into the muscle off the vertical it was commonly dislodged or bent. A second attempt at approaching the seal added greatly to the difficulties of the procedure. Gales and Burton (1987 a) suggested that a small barb on the needle would help alleviate this problem, though barbs have been considered unnecessary and their use in southern elephant seals has not been reported (Baker *et al.* 1988 and 1990).

Projectile syringes are also commonly used. Baker *et al.* (1988 and 1990) administered anaesthetics using pressurised projectile syringes, propelled through a blowpipe aimed at the posterior lumbar muscles. The technique used was based upon that used by Parry *et al.* (1981). Darts were made out of 10mL disposable syringes. The force which injected the drug was provided by a drop of liquid butane in a space behind the plunger. They were delivered by blow-pipe (a 1.5 m length of the appropriate internal diameter PVC tube). No barb was required. A 10 cm, 16 G needle whose end was blocked with epoxy and which had a small hole drilled into the side 1 cm from the tip was used. The hole was covered by a short length of tygon tube which slid clear as the needle entered the animal. Parry *et al.* (1981) found that the application of lubricant, such as petroleum jelly, to the outside of the needle was essential to ensure that the plastic tubing slid along the needle as it entered the animal.

Tranquillizer rifles have been associated with problems of skin penetration (Cline *et al.* 1969, Vergani 1985) and drugs being injected into fat, with prolonged or variable effects (Gales 1989). Vergani (1985) also found that wind affected dart accuracy.

Long, large bore needles are used to administer drugs. The needles have to be long enough to pass through the over-lying blubber and penetrate the muscle mass below and of sufficient internal diameter to allow rapid drug administration. Various needle sizes have been used ranging from 90 to 150 mm long, and 13 to 18 G (Bester 1988, Baker *et al.* 1988, Woods *et al.* 1989, Baker *et al.* 1990, Mitchell and Burton 1991). The most commonly used sizes are 100 mm, 16 G (Baker *et al.*

1988 and 1990) or 90 mm, 18 G (Gales and Burton 1987 a, Woods *et al.* 1989, Mitchell and Burton 1991). Needles for projectile use have been prepared after the technique of Parry *et al.* (1981) previously described (Bester 1988, Baker *et al.* 1988 and 1990).

Accidental intravascular administration has been reported when attempting to give drugs intramuscularly. Gales and Burton (1987 a) believed it led to the death of one animal, however Baker *et al.* (1988) believed they inadvertently administered drug intravascularly with no adverse effects. Ling *et al.* (1967) described spectacularly sudden collapse in animals to which suxamethonium was administered intravenously.

Intravenous administration

i. General

Intravenous administration requires the seal to be well restrained. In the field situation it is limited in its usefulness to pups, weaners and small yearlings and for administering further drug to adjust or deepen the level of chemical restraint. Its advantage is that drugs can be given until the desired effect is seen (to effect).

In southern elephant seals anaesthetic agents have been injected into the extradural intravertebral vein (Griffiths 1980, Gales and Burton 1987 a, Baker *et al.* 1990).

ii. Extradural intravertebral vein

The extradural intravertebral vein of southern elephant seals lies within the vertebral canal of the lumbar vertebrae (Hubbard 1969, Ridgway 1972, St. Pierre 1974, Ronald *et al.* 1977). Geraci and Sweeney (1978) have described venipuncture of the extradural intravertebral vein in phocid seals. The subject was placed in ventral recumbency. Using firm digital pressure along the dorsal midline of the lower lumbar area, the space between any two vertebral spinous processes was palpated. With the probing finger as a guide, a needle was inserted between the processes and pushed down into the extradural intravertebral vein. Sometimes there was a feeling of some release in pressure as the vein was entered. The extradural intravertebral veins overlie the cauda equina of the spinal cord. Excess probing in the area can lead to hind limb paralysis.

Arterial cannulation has been reported in northern elephant seals (Van Citters *et al.* 1965). The peripheral arteries and veins in elephant seals have not been described in detail.

iii. Plantar venipuncture

There are no reports in the literature of plantar venipuncture in elephant seals, however a blood sampling technique for the hind flipper of phocid seals has been described which could probably be used in southern elephant seals. A 32 mm, 18 G needle was introduced into the ventral (plantar)

aspect of the flipper, directly over DII or medial to DIV, at the level of the origin of the interdigital webbing. The needle was directed at a 10^{o} to 20^{o} angle from the plane of the flipper and inserted until either the main vessel or the complex vascular rete was struck. The blood was often mixed arterial and venous. Blood was described as continuing to flow freely following sampling and this was controlled by firm pressure (Geraci and Sweeney 1978).

Inhalation administration

i. General

There are no reports of induction or maintenance of anaesthesia in southern elephant seals using gaseous agents. However, two northern elephant seals have been anaesthetised using gaseous anaesthesia. Hamlin *et al.* (1972) used halothane to anaesthetise a 165 kg animal. The technique used was not clear, however reference was made to anaesthesia of the californian sea lion (*Zalophus californianus*) (Ridgway *et al.* 1969). In this technique the seals were restrained in a crush cage then strapped down. They were then premedicated with atropine (1/60th gr. per 50 kg sea lion), a glass hood placed over their head, and halothane introduced through a vaporizer at 10%. Induction occurred in 5 to 20 min. Once anaesthetised the hood was removed, the animal intubated, the vaporizer setting reduced to 2.5 to 3% and lowered as clinical signs indicated. Halothane concentrations of 0.75 to 1.5% were required to maintain anaesthesia. Recovery occurred within 1h or less post surgery. Hammond and Elsner (1977) induced anaesthesia in a 120 kg animal using an anaesthesia box and 80% nitrous oxide, 5% halothane and 15% oxygen. Induction occurred in 20 to 40 min. Evaluation of depth of anaesthesia was difficult because the animal was not accessible for examination. Also, premature attempts to intubate resulted in loss of anaesthesia gases and extended the induction period. Intubation with 1 to 2% halothane and oxygen maintained anaesthesia. Low ambient temperatures and reduced vaporization made inhalation induction difficult and prolonged and moisture can freeze movable parts in the expiratory circuit. It was considered desirable to have a rapid induction or immobilization so the seal can be placed in a warm field laboratory.

Hammond and Elsner (1977) described three techniques for induction with gas:

- Placing the anaesthetic mask directly over the nostrils.
- Placing the seal in an anaesthetic box.
- Immobilization with a muscle relaxing drug followed with intubation and artificial ventilation.

Induction with oxygen and 5% halothane and a mask was successful for light levels of anaesthesia. Physical contact of the mask on the nostrils increased the frequency of breath-holding. Partial withdrawal of the mask diluted the vaporized anaesthetic with atmospheric air and prolonged the induction phase.

Volatile anaesthetic agents such as halothane and isoflurane have been used for anaesthesia of other pinniped species (Gales 1989). Intubation and maintenance using positive pressure ventilation has been recommended though is not always essential as pinnipeds maintain some unconscious control of respiration (Sweeney 1974, Geraci and Sweeny 1978, Gales 1989). However, periods of apnoea are common (Hammond and Elsner 1977) and assisted ventilation helps control the P_{CO2} which commonly rises during anaesthesia (Geraci and Sweeney 1978). Hammond and Elsner (1977) used an apneustic plateau during ventilation of "large phocids" with a Bird 9 ventilator but suggested that there was no clear indication that such equipment was required. Phocids often require chemical induction prior to tracheal intubation because they can breath-hold during gaseous induction (Ridgway and Joyce 1975). McDonell (1972) overcame the problem of breath-holding by harp seals during induction by using a vaporizer that was able to deliver concentrations of halothane as high as 30%, thus making only a few breaths necessary for induction.

The main advantage of inhalation anaesthesia is that it is relatively safe. There is good control over the duration of anaesthesia and positive pressure ventilation can be provided in some cases if breathing ceases. Its use needs to be examined in southern elephant seals.

ii. Intubation

The anatomy of the larynx and upper respiratory tract of elephant seals has not been described. Pierard (1969) described the larynx of the Weddell seal (*Leptonychotes weddelli*; another phocid seal). He stated that the diameter of the glottis was about 25 mm. King (1983) described the pinniped larynx as having the normal number of cartilages that compose the mammalian larynx, with individual differences in detail in the various seals. The close approximation of the flat medial surfaces of the two arytenoid cartilages, which then abut against the posterior surface of the epiglottis, provide a tight seal against the entry of water into the trachea, and the powerful muscles of the larynx also help to keep this entrance closed (Schneider 1962 and 1963, King 1969 and 1972, Pierard 1969).

Hammond and Elsner (1977) reported that intubation of large phocids can be difficult, because apnoea can occur before relaxation of the jaw muscles.

Southern elephant seals have been intubated (Baker *et al.* 1990) though few details as to the technique or endotracheal tube sizes required were given. Baker *et al.* (1990) stated that if a prolonged apnoea occurred or if there were signs that the animal was becoming anoxic, as indicated by colour changes in the gums, tongue and conjunctiva, the animal was intubated with a cuffed veterinary endotracheal tube and ventilated by mouth. During apnoea, the larynx was usually found to be clamped tightly shut and the tube was placed in the trachea by inserting two fingers between the cartilaginous ridges of the larynx in spasm, and sliding the tube into the trachea between the 2 fingers.

A description of the technique for intubation and endotracheal tube sizes suitable for southern elephant seals is required.

Other routes of administration

i. Intrapleural

Bryden (1971 a) stated that, once immobilised, further drug can be given intramuscularly, intravenously or intrapleurally. However, few details were given regarding the intrapleural route.

ii. Intralingual

Baker and Gatesman (1985) stated that in apnoeic grey seals the pressure and rate of flow in the extradural intravertebral vein is very low, as part of the adaptations to anoxia during diving, and in many animals no or minimal response was seen to narcotic antagonists administered into this vessel. They considered that if the animals were apnoeic and unresponsive it was necessary to get the antagonist into circulation as quickly as possible. They administered drug into the tongue in the belief that this was a site where circulation was maintained during apnoea. Gales (1989) cautioned that injections given into the tongue may be painful and could affect feeding. However, Baker and Gatesman (1985) noticed that in the breeding season animals are not feeding so that any soreness produced would not have affected eating. One animal anaesthetised a week after receiving an injection into the tongue had only a small area of bruising. However, bruising of the tongue is probably a minor consideration when attempts are being made to resuscitate an animal severely depressed by anaesthesia.

iii. By mouth

Phencyclidine was administered to a young male southern elephant seal in a late stage of moult by squirting the drug above the tongue of the seal when it opened its mouth (Ross and Saayman 1970). This appeared to have little effect.

Multiple immobilizations of individual animals

Multiple immobilizations of individual animals have been reported though few details were given (Gales and Burton 1987 a, Baker *et al.* 1988, Woods *et al.* 1989, Mitchell and Burton 1991). In several cases lower doses of anaesthetic than those used for the initial episode were required. Baker (1988) described a seal that had been anaesthetised with ketamine 8 times, each occasion requiring a quarter of the normal dose of ketamine. Mitchell and Burton (1991) used doses of 2.8 - 6.5 mg/kg ketamine for initial immobilization and 2.1 - 4.5 mg/kg for repeat episodes. Gales and Burton (1987 a) administered similar doses to 6 animals without any apparent problems. Two seals were immobilized on 3 occasions and 1 of these required approximately twice as much ketamine for induction and maintenance as the remaining animals. Because both of these seals also exhibited

clinical disease as a possible result of multiple immobilizations (abscessation at injection site), they concluded that prolonged anaesthesia on more than 2 occasions was contraindicated, if the intervening period was relatively short. Fewer problems have been reported for less prolonged repeat immobilization procedures. Baker *et al.* (1990) also reported multiple immobilizations of single animals using tiletamine and zolazepam with no adverse side effects.

Anaesthetic drug pharmacokinetics have not been determined in southern elephant seals. A knowledge of the pharmacokinetics of anaesthetics in southern elephant seals might help determine guidelines for safe, repeated anaesthetic dose administration in this species.

Pre-anaesthetic and sedative medication

General

Premedication is not routinely performed during field immobilization. Premedicants, sedatives or co-anaesthetics are usually administered simultaneously with the anaesthetic. (Gales, 1989, has reviewed the anaesthetic drugs administered to northern elephant seals).

Atropine

A dose of 0.005 mg/kg has been recommended by Gales (1989), however this seems very low when compared with that used in other species of seal (0.02 to 0.04 mg/kg; Sweeney 1974, Hammond and Elsner 1977). Whether administration of atropine is necessary or effective at the 0.005 mg/kg dose is not known but this dose has been routinely administered to reduce salivation and bradycardia associated with anaesthesia in southern elephant seals (Gales and Burton 1987a, Woods *et al.* 1989, Mitchell and Burton 1991). Because of difficulties in estimating weights of animals prior to drug administration, female southern elephant seals have been given nominal doses of 2mL and males 4 mL of 0.65 mg/mL atropine sulphate intramuscularly combined with anaesthetic drugs (Woods *et al.* 1989). In other phocids it has been noticed that milk samples were difficult to collect after atropine administration and its use was suspended (Gales 1989). The effects of atropine on milk let down in southern elephant seals has not been described.

Tranquillizers and sedatives

i. General

The distinction between tranquillizers and sedatives is a question of semantics and of little value except that increased doses of tranquillizers tend to produce side effects but not loss of consciousness, whereas increased doses of sedatives produces more profound central nervous system depression that resembles anaesthesia (Gleed 1987). Tranquillizers administered to southern elephant seals include the phenothiazine promazine, and sedatives include the benzodiazepines diazepam and zolazepam, and the thiazine derivative xylazine.

ii. Promazine

Promazine has been used in southern elephant seals combined with phencyclidine (Table 2.1). Morgan *et al.* (1978) found that its addition to phencyclidine produced smoother recoveries than when phencyclidine was used alone. Cline *et al.* (1969) administered the drug intravenously to treat prolonged or clonic convulsions associated with phencyclidine use.

Promazine is no longer used as a coanaesthetic for immobilisation of southern elephant seals. Hubbard and Poulter (1968) stated that the effect of phenothiazine derivatives on pinnipeds was one of depression rather than tranquillization, and Sweeney (1974) and Haigh (1978) noted that the main contraindication was that they disrupted thermoregulatory mechanisms. There are, however, no published data on which to base these assumptions and further studies are required to determine the effect of phenothiazine derivatives on pinnipeds.

iii. Benzodiazepines

These drugs have anxiolytic and skeletal muscle relaxant effects and high margins of safety. The benzodiazepine is usually combined with a cyclohexamine to alleviate emergence reactions or improve skeletal muscle relaxation. Two benzodiazepines have been used in southern elephant seals: diazepam and zolazepam.

Diazepam has been shown to be useful when combined with ketamine (Gales and Burton 1987 a, Baker *et al.* 1988). It can be administered to pinnipeds orally, though this is not possible under field conditions. It has also been used intravenously in other pinnipeds to successfully facilitate minor procedures such as force feeding (Gage 1984), examination (Greenwood and Taylor 1983) and fibre-optic gastroscopy (Greenwood and Wild 1977). Hubbard (1969) suggested that diazepam was the best tranquilliser then available for phocid seals, though the assesment appeared subjective.

Use of zolazepam in combination with tiletamine has been described (Baker *et al.* 1990, Mitchell and Burton 1991). Though promising results were seen in initial work with this drug combination in other species of pinnipeds, including northern elephant seals (Hammond and Elsner 1977) and other animals (Stirling *et al.* 1985, Haigh *et al.* 1985, Stirling and Sjare 1988), 2 southern elephant seals of 5 to which this combined drug had been administered died in trials at Heard Island (Mitchell and Burton 1991). Despite these findings Baker *et al.* (1990) successfully immobilized a large number of southern elephant seals with this combination and stated that it appeared to be the best immobilizing agent available for use in this species. Comparative studies have not been performed in southern elephant seals, however they could help determine the most useful drug combination.

iv. Xylazine

Xylazine has been used alone (Vergani 1985) or combined with ketamine (Gales and Burton 1987 a, Bester 1988, Woods *et al.* 1989) to sedate southern elephant seals. Its use has been associated with vomiting, hyperthermia, excitement and deaths (Vergani 1985, Woods *et al.* 1989, Mitchell and Burton 1991). Anaesthetic reversal agents such as yohimbine and 4-aminopyridine are available for this group of drugs but have not been trialled in southern elephant seals. Woods *et al.* (1989) suggested that this drug might be implicated in initiating diving responses in immobilized animals.

v. Ketamine

Ketamine has been used as a sedative by Griffiths *et al.* (1979) who sedated immature males for transportation with 2.5 mg/kg administered intramuscularly.

Drugs used for chemical restraint or anaesthesia

General

A summary of drugs and dose rates used for chemical restraint or anaesthesia of southern elephant seals in presented in Table 2.1. The drugs used fall into 3 broad categories: neuromuscular blocking agents, barbiturates and cyclohexamines. Use of narcotic analgesics or gaseous anaesthetics have not been reported.

Neuromuscular blocking agents

Suxamethonium is the only neuromuscular blocking agent that has been used in southern elephant seals. It has been used alone (Ling and Nicholls 1963, Ling *et al.* 1967, Griffiths *et al.* 1979, Condy 1980) and combined with pentobarbitone (Bryden 1971 a, Griffiths 1980).

Ling *et al.* (1967) described a characteristic sequence of muscle paralysis associated with its use:

1. a rippling shudder along the length of the seal;
2. limpness of the hind flippers and then the fore flippers;
3. sagging of the lower jaw;
4. protrusion of the tongue;
5. passage of the nictitating membrane across the eye;
6. intercostal muscular paralysis;
7. paralysis of the diaphragm with ensuing apnoea for a period of 5 to 10 minutes.

Recovery followed almost exactly the reverse order of events.

It was found that the recovery time could be calculated from the linear relation:

$$I = 0.75R - 2.75$$

Where I = immobililization time (minutes) and R = recovery time (minutes).

The relationship was not further defined statistically. However, this approach may be very useful in predicting response to various anaesthetic drugs but would require controlled dose response studies.

Ling *et al.* (1967) reported little difference in the dose required for immobilization and that which induced apnoea but found that at a dose rate of 2.0 mg/kg 95% of elephant seals would be effectively immobilised and none would die. Of the 95%, 10% would be immobilized for less than 6.5 minutes, 80% between 6.5 and 27 minutes, and 10% for more than 27 minutes.

Despite satisfactory immobilization being reported in some cases, variable results, apnoea, fatalities, or the need for additional physical restraint have been reported in all studies using this drug. It has been suggested as causing extreme fright in other animals (Lumb and Jones 1984 p344) and the use of neuromuscular blocking drugs without any anaesthesia is now generally regarded as inhumane (Gales 1989). For these reasons suxamethonium is no longer used as the sole agent for chemical restraint.

Non-depolarising neuromuscular blocking agents have not been administered to southern elephant seals but have been used to immobilize a variety of other pinnipeds (Gales 1989).

Despite the difficulties, neuromuscular blocking drugs are useful in both human and veterinary anaesthesia and could be used to improve the balance of anaesthesia, facilitate positive pressure ventilation, or for treatment of seizuring in southern elephant seals.

Barbiturates

Backhouse (1964) suggested that, because of the depression of the cardiovascular and respiratory systems, pinnipeds were fairly sensitive to this group of drugs and recommended that they should only be used to induce anaesthesia and that other drugs should be used for maintenance. However, dose-response and comparative pharmacodynamic studies have not been performed and there appear to be few data to support these statements. This appears to be a common problem in reviewing the literature on pinniped anaesthesia; conclusions are usually based on the results of uncontrolled studies using small sample sizes. These conclusions appear to become ingrained in the literature after a number of years until accepted as fact. Controlled studies of drug usage are indicated in all species.

Barbiturates have been used less frequently than neuromuscular blocking agents or cyclohexamines for immobilization. The high pH of these solutions is associated tissue damage when injected

perivascularly, and problems of absorption following intramuscular administration (Lumb and Jones 1984) preclude them from use as an initial immobilizing agent in unrestrained animals. Only pentobarbitone has been used, to induce anaesthesia preceding euthanasia (Bryden 1971 a, Griffiths 1980). Prior immobilization with suxamethonium was used by both authors to allow drug to be administered either intravenously into the extradural intravertebral vein (Griffiths 1980) or intrapleurally (Bryden 1971 a). Concentrated solutions were used. Bryden (1971 a) administered a solution of 5 g/mL to a roughly third stage anaesthesia and Griffiths (1980) induced anaesthesia using a 325 mg/ mL solution at approximately 20 mg/kg (6 mL/ 100 kg). The authors did not discuss the effects of these drugs on the animals.

Gales (1989) reviewed barbiturate use in other seals and concluded that their unwanted side effects (such as cardiovascular and respiratory depression), the need for intravenous administration and the advent of more appropriate drugs meant that they were rarely used.

Despite the reported problems, barbiturates are a commonly used group of drugs, many veterinarians are familiar with their use and they may be useful if suitable doses could be determined. However, techniques to allow intravenous access other than premedication with neuromuscular blocking drugs (above) would need to be developed before they could be administered to wild animals.

Cyclohexamines

i. General

Cyclohexamines is a common term used to refer to the dissociative anaesthetics which are a group of chemical compounds that are capable of producing a state of anaesthesia during which the subject has an apparent dissociation from the environment (Short 1987 b).

The two cyclohexamines which are of clinical importance in elephant seals are ketamine and tiletamine; phencyclidine is of historic interest. Phencyclidine was the first to be used as an anaesthetic; the latter drugs are analogues of it. These three injectable anaesthetics have been the drugs most widely used for pinniped immobilization (Gales 1989). Cyclohexamines have been used alone, however they are usually used in combination with other drugs (parasympatholytics or sedatives) to diminish unwanted side effects such as poor muscle relaxation, salivation and emergence delirium (Short 1987 b).

ii. Phencyclidine and phencyclidine-promazine

Phencyclidine, one of the original dissociative anaesthetics, revolutionized chemical restraint and handling of animals (particularly nonhuman primates). However, as a result of its drug abuse potential it was withdrawn from the veterinary market. The development of other cyclohexamines having fewer undesirable side effects (Short 1987 b), has precluded this drug from further use.

Phencyclidine has been used alone (Ross and Saayman 1970, Morgan *et al.* 1978) or with promazine (Cline *et al.* 1969, Morgan *et al.* 1978) to immobilize southern elephant seals. When used alone prolonged recovery, with animals being aware of their surroundings while still paralysed, has been described (Morgan *et al.* 1978). The addition of promazine was reported to give a smoother and more rapid recovery from immobilization (Morgan *et al.* 1978). Cline *et al.* (1969) also used promazine with phencyclidine and found that reaction to the drugs followed a characteristic and predictable sequence of events. The animal exhibited progressive ataxia and loss of coordination accompanied by increased salivation and watering and rolling of the eyes. Respiration was noticeably slowed during the inspiratory phase. Minor tremors (tonic convulsions) frequently became evident in the neck region, and along the tail and hind flippers. Whenever prolonged or clonic convulsions appeared, additional promazine was given.

iii. Ketamine

Ketamine has been used for chemical restraint of southern elephant seals (Morgan *et al.* 1978, Griffiths *et al.* 1979, Ryding 1982, Woods 1991). There is little information available on its usefulness and effects in southern elephant seals though it has been widely used in a number of other pinniped species (Gales 1989). The few southern elephant seals to which it has been administered were handled relatively easily and safely and there was little change in heart rate, respiratory rate, or rectal temperature (Ryding 1982). Hyperthermia has not been associated with its use as it has been in other seals (Sweeney 1985), however tremoring, wide individual tolerance, lacrimation (Ryding (1982) and deaths (Woods 1991) have been reported.

It was found that the addition of a sedative such as diazepam or xylazine masked some of the undesirable effects of ketamine in other seals (Geraci *et al.* 1981) and thus ketamine is no longer used alone for initial immobilization of southern elephant seals. Comparative studies to determine the most suitable sedative to combine with ketamine have not been performed. Gales and Burton (1987 a) found that diazepam and xylazine were both suitable combinative drugs with ketamine; neither appeared superior, with both effectively preventing the reported side effects of ketamine. However Baker *et al.* (1988) suggested that ketamine combined with diazepam provided the best and safest anaesthetic combination then available. This combination caused fewer adverse side effects than ketamine and xylazine although severe respiratory depression was sometimes seen with either sedative.

Ketamine alone has been used to prolong chemical restraint (Gales and Burton 1987 a, Mitchell and Burton 1991).

iv. Ketamine and diazepam

Ketamine and diazepam have been used to chemically restrain southern elephant seals (Gales and Burton 1987 a and b, McCann *et al.* 1989, Baker *et al.* 1988, Woods 1991). The drugs have been administered in various ratios (Baker *et al.* 1988, 20:1; McCann *et al.* 1989, 50:1; Woods 1991, 100:1; Gales and Burton 1987 a, not stated). It is not known which of these ratios, and what dose rate, is most appropriate. However, Baker *et al.* (1988) suggested that 6 mg/kg ketamine and 0.3 mg/kg diazepam provided the best and safest anaesthetic combination then available for immobilizing southern elephant seals in the field. Gales and Burton (1987 a) used a combination of ketamine (5.4 - 14.95 mg/kg) and diazepam (0.03 - 0.11 mg/kg) and, despite one fatality, considered that results were generally "reliable".

v. Ketamine and xylazine

Ketamine and xylazine have been used to immobilize southern elephant seals (Gales and Burton 1987 a, Bester 1988, Woods *et al.* 1989, Mitchell and Burton 1991). Reports vary as to its usefulness. Gales and Burton (1987 a) found it produced good immobilization and Bester (1988) believed it to be relatively safe and effective, however Woods *et al.* (1989) and Mitchell and Burton (1991) reported deaths and complications such as vomiting and hyperthermia. Mitchell and Burton (1991) suggested that the fatalities were due to regurgitation and aspiration of stomach contents associated with anaesthesia and stomach lavaging and concluded that the xylazine component was responsible.

vi. Tiletamine and zolazepam

Tiletamine is a more recent analogue of phencyclidine. It has been administered to southern elephant seals combined with the sedative zolazepam (Baker *et al.* 1990, Mitchell and Burton 1991). Reports of its usefulness for immobilization vary. Two of 5 southern elephant seals died when administered doses that were successful in polar bears (Mitchell and Burton 1991). However, Baker *et al.* (1990) successfully immobilized 90 southern elephant seals (including 3 adult males) a total of 195 times using a combined dose of 1 mg/kg of tiletamine and zolazepam with no fatalities. Recovery time, though not recorded, was thought to be faster than that after ketamine based drug mixtures. Six animals became apnoeic but recovered. It was thought that the effects of the tiletamine and zolazepam mix were more predictable than those of other preparations and the authors stated that it appeared to be the best immobilizing agent currently available for use. However, comparative studies have not been conducted.

Narcotic analgesics

Narcotic analgesics have their action in the central nervous system where they alleviate pain and cause some degree of central nervous system, cardiovascular and respiratory depression (Gales 1989). Their use in other seals has been reviewed by Gales (1989) who concluded that the associated fatalities and reports of apnoea probably preclude them, and their reversal agents, from use in pinnipeds. It was

recognised, however, that the ability to reverse these agents was an advantage. Despite these conclusions the fact remains that no dose-response or other objective studies have been performed in pinnipeds using these agents. These drugs may be very useful and further studies using them are indicated.

Inhalation anaesthetics

Inhalation anaesthetics have not been administered to southern elephant seals. Their use in other seals has been reviewed by Gales (1989) who commented that despite their advantages the high level of expertise required and financial and logistical restraints often prevent their use in the field. It is, however, more likely that their use has been limited simply because they have been unnecessary. Field procedures are usually of short duration and only low levels of restraint are required; criteria which can now be achieved using injectable agents.

Anaesthetic reversal agents

General

Various antagonists are available for some of the drug combinations used to chemically restrain southern elephant seals, however none have been administered. Those most commonly used in other animals include: 4-aminopyridine and yohimbine for ketamine and xylazine or xylazine; flumazenil, sarmazenil and 4-aminopyridine for benzodiazepine/ cyclohexamine based combinations; and naloxone, naltrexone and diprenorphine for narcotic based combinations. The analeptic doxapram has also been used nonspecifically to cause arousal.

Yohimbine

Yohimbine, an α_2 adrenergic antagonist, has been used in preliminary trials in Hooker's sea lions. Results suggested that, while it effectively antagonised some of the actions of a ketamine and xylazine combination, its administration often caused violent tremors and convulsions (Cawthorn 1989). Gales (1989) suggested that the yohimbine was primarily antagonising the xylazine, which was then failing to mask the side effects of ketamine. He concluded that further trials were required to properly determine the efficacy of yohimbine in pinnipeds.

Narcotic reversal agents

See Narcotics.

Administration of additional drugs

Administration of additional drugs has been by intramuscular, intravenous or intrapleural injection. By necessity, animals that failed to become adequately immobilized after the initial dose of drug have had further doses administered intramuscularly. Operators have usually waited for periods of 10 to 20 minutes before administering additional drug (Ryding 1982, Gales and Burton 1987a). Once immobilized, further drug has been administered intravenously or intramuscularly to prolong or increase the level of chemical restraint. Despite the reported advantages of intravenous drug administration, including lower doses, more rapid induction and good control over the duration and degree of immobilization (Englehart 1977), most authors have given further doses of drug intramuscularly. Gales and Burton (1987a) found that duration of effect was reduced and response to injection less predictable when additional ketamine was administered intravenously rather than intramuscularly and, because these animals were required to be immobilized for prolonged periods, these workers administered further drugs intramuscularly. However Baker *et al.* (1990) used intravenous administration of small volume increments of tiletamine and zolazepam when deeper levels of chemical restraint were required. This subsequent dose took effect in 15 to 30 seconds and thus could be used to very accurately adjust the degree of immobilisation. Guidelines for additional drug administration were not presented; presumably they were given to effect.

Specific recommendations have not been made regarding additional doses but they have, however, been reported (Table 2.1). Ryding (1982) administered estimated doses of 1.58 mg/kg and 2.93 mg/kg of ketamine intramuscularly to 2 male southern elephant seals that had received an estimated 4.48 mg/kg and 4.63 mg/kg ketamine 20 minutes before but had failed to become immobilised. The animals were immobilised 7 and 11 minutes later. Bester (1988) administered further ketamine (1.56 and 1.18 mg/kg) and xylazine (1.20 and 1.13 mg/kg) to 1 post- and 1 pre-partum female to which ketamine (4.92 mg/kg) and xylazine (0.83 mg/kg) had been administered. Immobilization was achieved within 4 minutes. Gales and Burton (1987 a) reported doses of drugs used per h to maintain immobilization and Baker *et al.* (1988 and 1990) reported cumulative doses of drugs used to immobilize animals to which more than 1 injection had been performed. Mitchell and Burton (1991) administered additional doses of approximately 25% of the initial dose of ketamine intramuscularly to induce or maintain anaesthesia.

Additional doses of 500 mg ketamine have been administered to northern elephant seals to maintain restraint for up to 4 h (Costa *et al.* 1986). The route of administration was not stated.

Unwanted effects of anaesthetic agents

Musculoskeletal system

Tremor, ranging from very slight to tonic convulsions, has been associated with cyclohexamine administration in southern elephant seals (Cline *et al.* 1965, Morgan *et al.* 1978, Ryding 1982, Gales and Burton 1987 a, Bester 1988, Mitchell and Burton 1991). Tremor is alleviated to some degree by use of sedatives (Gales 1989) administered with the cyclohexamine (Gales and Burton 1987 a) or after its onset (Morgan *et al.* 1978).

Special senses

i. Ophthalmic signs

There is little information on the effect of anaesthetic agents on ophthalmic signs in elephant seals. Cline *et al.* (1969) reported rolling of the eyes in animals to which promazine and phencyclidine had been administered. Ryding (1982) observed that corneal reflex was present at maximum immobilisation in each of the 5 animals he anaesthetised with ketamine. "Apparently enlarged pupils" were noticed by Ross and Saayman (1970) in a young bull immobilized with phencyclidine. The eyes also remained open.

ii. Lacrimation

Ryding (1982) observed an increase in lacrimation in 3 of 5 seals anaesthetised with ketamine but it was considered of doubtful significance as eye swabs were taken in all cases. Cline *et al.* (1969) also reported increased lacrimation associated with phencyclidine use.

Central nervous system

There is little information available on the central nervous system effects of anaesthetic agents in southern elephant seals. Absolute or relative drug overdose have been implicated as cause of death in several animals (below).

Vergani (1985) reported that the deaths of two pups to which an estimated 4:4 and 6.3 mg/kg xylazine had been administered were associated with drug overdose. Though these doses are lower than those used for immobilization of some other animals (Booth 1982 c, range 0.5-10 mg/kg) they are higher than those used for immobilization of other phocid seals (Erickson *et al.* 1974, Vergani *et al.* 1986; range 2-3 mg/kg).

Gales and Burton (1987 a) suggested that an animal in their study died as a result of intravascular administration of 8.20 mg/kg ketamine and 0.04 mg/kg diazepam and thus relative drug overdose. However, Baker *et al.* (1988) believed they administered ketamine and diazepam intravascularly to two animals; in both cases the induction time was less than one minute but no adverse effects were

seen. Baker *et al.* (1988) did not state the doses of drugs administered to each animal which may have been lower, or less rapidly administered, than those admistered by Gales and Burton (1987a).

Digestive system

i. Salivation

Increased salivation has been noticed using several cyclohexamine based drug combinations (Cline *et al.* 1969, Ryding 1982). Neither of these authors administered a parasympatholytic drug.

ii. Vomiting

Vomiting or retching has been associated with immobilization of southern elephant seals. It has usually been associated with gastric lavage or the use of xylazine (Vergani 1985, Mitchell and Burton 1991) and has been suggested as the cause of fatalities due to aspiration of stomach contents (Mitchell and Burton 1991). Ling *et al.* (1967) noticed retching in animals immobilized with suxamethonium but they believed that the fasting habits of the animals while ashore possibly saved them from complications arising from regurgitation of stomach contents. Mitchell and Burton (1991) described regurgitation and aspiration of stomach contents in animals anaesthetised with ketamine and xylazine. They suggested that the combination of anaesthesia and stomach lavaging were probably responsible, although one seal vomited before intubation. They also suggested that regurgitation and aspiration of stomach contents in several species of seal was associated with the administration of xylazine.

iii. Gastric lavage

Gastric lavage whilst animals were immobilized with ketamine and xylazine has been reported (Mitchell and Burton 1991). It was associated with regurgitation, vomiting, respiratory distress, and two deaths. Intubation was not used.

iv. Defecation

The effects of anaesthetic agents upon the gastrointestinal system and defecation of southern elephant seals have not been discussed. Baker *et al.* (1990) reported that grey seals frequently defecated large volumes after immobilization with ketamine based mixtures, making handling the animals unpleasant, and, as the hind flippers were frequently contaminated, blood sampling and tagging carried a higher risk of infection. With tiletamine and zolazepam animals did not defecate. It was not known whether the ketamine based mixtures caused defecation or whether tiletamine and zolazepam inhibited it.

Respiratory system

Normal respiratory rates and patterns have been described for wild free-ranging southern elephant seals on land (Kenny 1979). Animals were described as showing some form of rhythmic variation of

breathing rate characterised by some minutes of eupnea followed by a period of apnoea of variable duration. There is little information available on respiratory rate and pattern for immobilized southern elephant seals. Cline *et al.* (1965) noted that respiratory rate was noticeably slowed during the inspiratory phase with phencylidine use. There was little change in respiratory rate in five animals immobilised with ketamine (Ryding 1982). Bull respiratory rates ranged from 3.5 to 4 breaths/min, the adult female 4 breaths/min, and the first year female pup 5.5 - 7 breaths/min. Of five pre and post-partum cows anaesthetised with ketamine and xylazine by Bester (1988), four breathed regularly throughout immobilisation whilst one had a 20 minute period of apnoea, but resumed regular breathing 5 minutes after administration of doxapram, the effect of which was equivocal. Mitchell and Burton (1991) described respiratory rates of from 4 to 20 breaths/min for animals immobilized with ketamine and xylazine that were breathing regularly.

Apnoea, laryngeal spasm and closure of the nostrils have been reported with a variety of anaesthetic agents (Vergani 1985, Baker *et al.* 1990, Mitchell and Burton 1991). Apnoea is common, has been associated with death and is often seen upon induction, after additional drug administration, or associated with gastric lavage or the use of suxamethonium (Ling and Nicholls 1963, Condy 1980, Mitchell and Burton 1991). Its duration varies but periods of up to 60 minutes have been reported (Mitchell and Burton 1991). The total period of apnoea does not appear to be correlated with dose of drug administered, however Mitchell and Burton (1991) reported that duration of anaesthesia increased with length of apnoeic period.

Ling *et al.* (1967) described the recovery from apnoea associated with the use of suxamethonium. Over a period of 4 - 9 minutes following apnoea, breathing was initially slow and irregularly at first (6 breaths/ minute), becoming rapid and more regular (18 - 22 breaths/ minute), and then slowed again slightly. However (Ling *et al.* 1967) considered normal respiration to be so irregular while the animals were sleeping that a basal rate was difficult to determine and considered it somewhat meaningless to compare with the hyperventilation response.

Comparative studies on the effect of anaesthetic drugs upon respiration have not been performed.

Cardiovascular system

It has been stated that elephant seals, because of their large size, are difficult to auscultate (Needham 1978). There is little information available on the cardiovascular response to anaesthetic agents of southern elephant seals. Normal resting heart rates on land have been described (Kenny 1979). They ranged from 96 beats/min for a 1 month old pup to 23 beats/min for a bachelor bull. Heart rates from pups (mean = 81 beats/minute) were significantly higher than those from other sections of the population (mean = 53 beats/minute). Heart rates of immature seals were higher than those of adults

with the exception of cows after weaning their pups. The differences between the rates observed from different adult groups were not significant, except for those cows at the end of lactation and the onset of the breeding season; these were significantly higher than other heart rates. Heart rates of females ranged from 40 to 62 beats/minute; for males from 36 to 54 beats/minute. Sweeney (1974) gave mean heart rate of captive elephant seals as 80 beats/minute.

Kenny (1979) found that observations at 5 minutes intervals on individual seals showed changes in heart rate of up to 10% without alterations in the activity pattern of the individual. No regular pattern of heart rate variability relative to breathing rhythms was noted, although Bartholomew (1954) observed a 15% reduction of heart rate during apnoeic periods for northern elephant seals. Further, differences in heart rate before and after periods of apnoea, similar to those recorded during experimental dives of other seal species (Harrison and Kooyman 1968) were not observed.

There was little change in heart rate in five southern elephant seals immobilised with ketamine (Ryding 1982). There was no suggestion of pathological cardiac arrhythmia. Heart rate of the three immobilised bulls ranged from 34 - 54 beats/min, the adult female 72 - 76 beats/min, and the first year pup 72 - 76 beats/min. Ryding (1982) also used a medical sphygmomanometer to measure arterial blood pressure on the front limb of a first year pup immobilised with ketamine alone. Repeated estimates gave systolic pressures of 110 - 115 mmHg, and diastolic pressures of 60 - 85 mmHg. It was not possible to use this technique on larger seals.

Cardiovascular dynamics have not been been studied in southern elephant seals but have been in northern elephant seals during studies of simulated diving in a tank after immobilization with phencyclidine (Van Citters *et al.* 1965). Blood pressure was sampled via polyethylene tubes inserted into the aorta via flipper arteries. Aortic blood pressure averaged 120/80 mmHg, and excursions as wide as 50 mmHg occurred during respiration. Resting heart rates ranged between 50 and 85, and averaged 60. The maximum heart rate during exertion or after atropine was < 100. During the longest dive, which was 40 minutes in duration, the heart rate reached 4. No electrocardiographic abnormalities were recorded during diving activity. Aortic blood pressure was maintained or was slightly elevated during diving bradycardia. Iliac artery blood flow was drastically curtailed, usually to 25% of resting levels immediately after the onset of diving, and commonly fell to 0 in dives lasting longer than 10 minutes. Carotid artery flow fell in proportion to the heart rate, but the velocity and volume of blood pumped into this vessel with each beat was only slightly reduced. It was concluded that these changes implied that intense vasoconstriction occurred in skeletal muscle bed, but was less marked in the distribution to the carotid artery. Diving bradycardia was abolished by atropinization of the animal and bradycardia and vasoconstriction identical with that developed during diving was provoked by electrical stimulation of the periventricular nucleus in the anterior hypothalamus. The resting ECGs were essentially identical in configuration to their counterparts recorded in common

laboratory animals or man. All these factors, but in particular heart rate, may have been influenced by anaesthesia (Van Citters *et al.* 1965).

Thermoregulatory system

Despite the generally accepted risk of hyperthermia associated with pinniped chemical restraint (Gales 1989), with the exception of xylazine and combinations containing xylazine, rectal temperatures reported during anaesthesia (35.8-38.8^{o}C; Ryding 1982, Mitchell and Burton 1991) compare favourably with those presented for non-chemically restrained animals (34.8-38.4^{o}C; Laws 1956, Kenny 1979). Cline *et al.* (1969) suggested that the apparent lack of hyperthermia in their study was associated with the relatively cool temperatures under which animals were immobilized.

Hyperthermia, or clinical signs interpreted as indicating hyperthermia, have been associated with deaths in southern elephant seals to which anaesthetic agents have been administered (Vergani 1985, Woods *et al.* 1989). Excluding one animal suspected of dying from hyperthermia, Vergani (1985) reported temperatures of weaned pups immobilised with xylazine of approximately 35.3^{o}C to 40.7^{o}C. The animal which was thought to have died from hyperthermia had a high initial rectal temperature (approximately 40.4^{o}C), which rose to 60.5^{o}C terminally. It was believed that the inability to resort to behavioural mechanisms of cooling whilst immobilised prevented heat loss and caused the animal's death. He recommended close monitoring of the temperature of immobilized animals and moistening with water if necessary. A decrease in body temperature after the effects of the drug had passed was seen in animals immobilised in Antarctica, and was believed to have been produced spontaneously, presumably due to the cold environmental conditions.

Despite cold predisposing other anaesthetised animals to hypothermia (Lumb and Jones 1984), complications associated with hypothermia have not been recorded. Gales and Burton (1987 a) found that despite immobilizing moulting animals for prolonged periods in temperatures as low as - 23^{o}C there were no signs of hypothermia. However, as body temperature was not monitored some change in core or skin temperature may have occurred. They suggested temperature monitoring should be considered in future studies and the effect of anaesthesia on thermoregulation in these animals needs to be examined.

Use of drugs in pregnancy

There is little information available on use of drugs in pregnancy in southern elephant seals. Southern elephant seals have a 12 month gestation period (including a 3 month period of delayed implantation), therefore most females which are immobilized are pregnant. Whether chemical restraint or anaesthesia affects the foetus or mother is unknown. Xylazine use has not been fully evaluated in pregnant animals. Evidence is conflicting; some evidence suggest that it may be

contraindicated, possibly predisposing to abortion (LeBlanc *et al.* 1984), other evidence suggests otherwise (Gleed 1987). Moreover, its use is usually avoided during the later stages of pregnancy. Bester (1988) administered ketamine and xylazine to 3 pre-partum cows which gave birth within a week to apparently healthy pups. Pups are usually born within a week of cows hauling out (King 1983). Bester (1988) did not speculate on the possible effects of these drugs on pregnancy, the effects of which need to be examined in southern elephant seals.

Complications associated with chemical restraint and anaesthesia and their prevention and treatment

General

During anaesthesia complications can occur even when optimal care is provided and generally involve one of the following categories: depth of anaesthesia, ventilation, cardiovascular function, or temperature regulation (Eicker 1986). Fatality rates and complications have been discussed (Gales 1989, Mitchell and Burton 1991). Apnoea, aspiration pneumonia, drug overdose, or dive responses have been implicated. In many cases the cause of deaths were unknown. Other problems include attack by other seals or conspecifics, desertion of pups (Woods *et al.* 1989), and going to sea and drowning (Mitchell and Burton 1991).

Monitoring

Various criteria and techniques have been used for monitoring southern elephant seal anaesthesia and chemical restraint. However, even the most fundamental of parameters can be difficult to assess. The thorax is difficult to auscultate and peripheral pulses are only palpable in young animals (Needham 1978, Ryding 1982). Preference for light planes of anaesthesia or sedation, rapid recovery and performance of non-invasive procedures of short duration using injectable anaesthetic agents, limits the time available for placement of monitoring equipment. The inaccessibility of peripheral arteries and central venous access coupled with difficulties in supplying power, the delicate nature of some equipment and lack of personnel, have made routine monitoring of blood pressure, central venous pressure, blood-gases and ECG impractical for southern elephant seals anaesthetised for short periods in the field.

For these reasons there is little information in the literature on monitoring of anaesthetic depth and the response of southern elephant seals to anaesthesia. This lack of information compounds the problems of monitoring anaesthesia and is further exacerbated by a lack of information regarding normal values whilst under anaesthesia for heart rates, respiratory rates, rectal temperatures, biochemistry, haemotology, blood pressure, central venous pressure, blood gases and ECG.

Cline *et al.* (1965) determined degree of paralysis by touching the tail and hind flippers of immobilized southern elephant seals. This often elicited minor convulsive jerks of the flippers or head or body muscles, but responsiveness gradually subsided until the face could be touched without eliciting a response. The time interval between initial drug injection and immobilization was recorded as the latent period. They did not have sufficient time to observe the animals for more than 2 h 40 minutes so no other parameters were presented.

Ryding (1982) defined induction time, duration of maximum immobilization and total immobilization time for southern elephant seals as follows.

- Induction time was measured from the start of the injection until the seal ceased to raise its head when approached.
- Duration of maximum immobilization was defined as the time between induction and the onset of uncoordinated provoked voluntary movement.
- Total immobilization time was defined as the time from induction until the seal was able to raise its head when approached.

It was found that in all cases muscle tone was present throughout immobilization; no cyanosis of mucous membranes was observed; corneal reflex was present at maximum immobilization; and a minimal withdrawal reaction to painful stimuli was observed in only one animal.

Vergani (1985), classified the response to different doses of xylazine administered to weaned pups according to the reaction to pain caused at the moment of blood extraction. The reactions were divided into three categories: without reaction; slight; strong. Gales and Burton (1987 a) recorded induction time as the period between injection and failure of the animal to raise its head when patted on the back. Duration of immobilization was recorded as the interval from the end of induction to the time when the animal regained the ability to resist being handled. Baker *et al.* (1988) recorded depth of anaesthesia on an arbitrary scale of 1 to 5, from a minor degree of sedation to surgical anaesthesia. A standard form was completed for each immobilisation, recording the animal's weight and dimensions, the times of procedures and details of the anaesthetic. Bester (1988) used Ryding's (1982) definitions for induction time, duration of immobilisation. He believed that responses to immobilization may vary with methods of recording the behaviour of the seals. Woods *et al.* (1989) defined sedative induction time as the interval from injection until the animal failed to respond to head patting. Duration of sedation was defined as the time from induction until the animal could raise its head.

Development of a standardised system of monitoring would allow more accurate assessment of the patient and comparisons to be made between anaesthetic agents.

Anaesthetic depth

i. General

There is little information available on the control of anaesthetic depth. Use of injectable anaesthetic agents, lack of anaesthetic reversal agents and the inability to treat all complications associated with chemical restraint have made control of anaesthetic depth difficult.

Problems of anaesthetic depth in southern elephant seals generally fall into three categories: inadequate depth, excessive depth and prolonged anaesthesia or recovery. Inadequate anaesthesia prevents completion of planned procedures and excessive anaesthesia can lead to various complications by depressing the cardiovascular and respiratory systems which may cause tissue or organ injury or death, depending upon the duration and degree of depression (Eicker 1986).

ii. Excessive anaesthetic depth

This has been associated with relative drug overdosage after suspected intravascular administration of anaesthetic drugs (Gales and Burton 1987 a) or absolute drug overdose (Vergani 1985). Increased anaesthetic depth, especially in combination with systemic or metabolic disorders has been associated with prolonged recovery in other animals which can lead to fatal respiratory depression or ventricular fibrillation (spontaneous or induced by hypothermia; Eicker 1986).

iii. Prolonged recovery

Bester (1988) stated that a rapid recovery was desirable to minimise the possibility of interference by conspecifics, and to promote rapid resumption of pup attendance by lactating cows. Prolonged recovery has been seen associated with anaesthetic and sedative use. Morgan *et al.* (1978) described recovery periods of from 65 minutes to > 9 h associated with phencyclidine use and considered this a disadvantage. Woods *et al.* (1989) described an animal sedated for 6 h that failed to recover and died 18 h after administration of ketamine and xylazine.

Respiratory system

i. General

The role of apnoea in complications associated with anaesthesia is not known. There is evidence to suggest that sleep apnoea in the northern elephant seal has physiologic aspects that are similar to those seen in freely diving seals (Castellini *et al.* 1986) and it is possible that circulatory changes consistent with those seen during diving occur in some anaesthetised southern elephant seals (Gales and Burton 1987 a). These changes have been termed "the dive response" (Hubbard and Poulter 1968).

ii. Apnoea and the dive-response

Pinnipeds have a respiratory centre that is relatively insensitive to the normal respiratory stimulant CO_2 (Hubbard 1969) and there is greater cerebral tolerance of low oxygen levels than is the case for

terrestrial species (Elsner *et al.* 1970). In southern elephant seals and other phocids, apnoea has been compounded by upper airway obstruction or closure of the nostrils, which is their normal relaxed state (Baker and Gatesman 1985). Hammond and Elsner (1977) noted that there was a marked difference in the level of anaesthesia that produces apnoea in terrestrial and aquatic mammals. It was their experience that apnoea occurred almost with the onset of induction and consequently it was found that a surgical plane of anaesthesia nearly always required intubation and resuscitation.

The major physiological changes associated with the dive response have been determined largely under laboratory conditions and include bradycardia, and a decrease in cardiac output and peripheral circulation (Scholander 1940, Elsner *et al.* 1966, Elsner 1969, Kooyman *et al.* 1981, Butler and Jones 1982). Metabolism is reduced in many tissues (Kooyman *et al.* 1981). Blood flow to the liver decreases and the blood is stored in large venous sinuses (Harrison and Tomlinson 1958). These actions conserve oxygen and glucose for the heart and central nervous system (Kooyman 1968, Robin *et al.* 1981). Respiratory acidosis during the dive and metabolic acidosis during recovery have been reported (Elsner 1969).

Blood returning to the heart via the caudal vena cava is restricted by the caval sphincter level with the diaphragm and pools in the inferior vena cava and hepatic sinus (Harrison and Tomlinson 1958). This system acts as a storage compartment for relatively well-oxygenated blood. In northern elephant seals approximately one fifth of the total blood volume can be contained within the inferior vena cava (Elsner 1969). In the latter portion of the dive, blood oxygen has been shown to be higher in the inferior vena cava than in arterial circulating blood (Elsner 1969). During the dive the caval sphincter opens periodically during diastole letting oxygenated blood enter the heart-brain circulation (Butler and Jones 1982).

Natural dives are usually short and aerobic (Kooyman 1981). The blood volume of the adult southern elephant seal is considerably higher than that of terrestrial mammals (207 ml/ kg compared with 80 ml/ kg in an adult human being; Bryden and Lim 1969) and the mean corpuscular volume is also larger (four times that of terrestrial animals in northern elephant seals; Wickham *et al.* 1989). Even when combined with low red cell counts this gives the northern elephant seal high packed cell volumes that can be twice those of terrestrial animals (Lane *et al.* 1972, Wickham *et al.* 1989). The larger blood volume, haemotocrit and mean corpuscular haemoglobin concentrations result in a six fold increase in blood oxygen capacity in the northern elephant seal compared with the pig (Wickham *et al.* 1989). In addition, the myoglobin content is higher than that of land mammals which affords the seal an even greater oxygen capacity (Robinson 1939). The combination of a large total oxygen store relative to body size, cerebral tolerance to hypoxaemia, avoidance of anaerobic diving and parsimonious use of blood oxygen due to the lowered energy requirements of various organs are

mechanisms by which the problems of hypoxia or hypercapnia are decreased (Elsner *et al.* 1970, Kooyman *et al.* 1980, Kooyman 1981, Hindell *et al.* 1992).

Two theories have been used in an attempt to explain deaths under anaesthesia associated with apnoea and the dive response. Mitchell and Burton (1991) proposed that the full physiological response associated with apnoea may have been affected by anaesthesia, resulting in early development of anaerobic conditions and death within the first h of anaesthesia. They also presented an alternative hypothesis (Backhouse 1964) that some of the anaesthetic was pooled in venous sinuses during "diving" and was not metabolized; when the animal "surfaced" to breath, the anaesthetic agent was released and entered the brain to cause a relapse into deeper anaesthesia.

What stimulates southern elephant seals to enter a dive response is unknown. Fright, anaesthetic drug effects, apnoea and upper respiratory tract obstruction, or normal physiological changes associated with sleep and breathing on land could all play a role or have been implicated (Backhouse 1964, Gales and Burton 1987 a, Woods *et al.* 1989, Mitchell and Burton 1991). The integration of reflexes involved and the relationships appear complex (see Figure 1, Blix 1987). The lack of knowledge of the cause of apnoea and diving-reflexes in anaesthetised southern elephant seals and the effect they have upon the anaesthetised animal have made pharmacological treatment and prevention of this problem unsuccessful. These theories appear complex in animals for which safe doses of anaesthetic drugs have not been determined. Problems may be as simple as drug overdose and studies of drug kinetics and circulation during apnoea are indicated.

iii. Prevention and treatment of apnoea and the dive-response

Prevention of apnoea and dive-responses has not been discussed. Van Citters *et al.* (1965) found that diving bradycardia was abolished in northern elephant seals by atropinization of the animal (the dose was not stated), however apnoea and dive-responses have been seen in southern elephant seals to which a total dose of 1.2 mg (for cows) or 2.4 mg (for bulls) atropine had been administered (Woods 1989, Woods 1991, Mitchell and Burton 1991; approximately 0.005 mg/kg). Presumably the dosage of this drug was inappropriate to prevent the response.

Various techniques, including use of respiratory analeptics and artificial ventilation, have been used in the treatment of apnoea and the dive response, however neither appear to be completely reliable.

In many respects the diving circulation in the seal is similar to that following circulatory adjustments accompanying early hypovolaemic shock in man and other animals (Ronald *et al.* 1977). It has been theorised that due to pooling of blood and slow flow rates in readily accessible vessels, drugs administered into the extradural intravertebral vein may not reach their central site of action, explaining reports of little effect in apnoeic seals (Baker and Gatesman 1985). It was believed that the

tongue was a site where circulation was maintained during apnoea (Baker and Gatesman 1985), however the respiratory stimulant doxapram has been administered to apnoeic animals by a variety of routes, including intralingually and had little or no effect (Mitchell and Burton 1991).

Positive pressure ventilation has been used more successfully (Baker *et al.* 1990), however problems have been associated with ventilation of large phocid seals. Hammond and Elsner (1977) stated that resuscitation in large phocid seals could be complicated by the flexible thorax. (How large "large" was was not stated, however weights were presented of 100 - 400 kg for Harbour and Weddell seals.) In phocid seals 10 of the 15 ribs articulate with the sternum. The weight of the thorax, therefore, comes to bear on the lung parenchyma which permits lung collapse when the seal is in a prone position and in the study of Hammond and Elsner (1977) the ventilation equipment had to inflate the lungs against the weight of the thorax. It was found that the inflatory pressure could exceed the bursting limits of anaesthetic systems which used a pneumobag for positive pressure. These authors reported that the problem could be eliminated by placing the animal on its back or side and using a resuscitator with a negative pressure expiratory phase to assist expiration in large phocids though no data were presented.

At present there appears to be no satisfactory, consistently successful pharmacological technique available to prevent or antagonise the dive reflex. Intubation and positive pressure ventilation appear to be more successful, however size of animals, logistics and other problems limit its use. Prevention of problems are also frustrated by the inability to use gaseous anaesthesia and the lack of understanding and control of the anaesthetic episode. The respiratory stimulant doxapram may offer an alternative in situations where positive pressure ventilation cannot be achieved, however, its efficacy and dosage have not been determined.

Attack by other seals or sympatric species

Woods *et al.* (1989) describe the death of a cow that had its skull crushed by a bull during recovery from anaesthesia. It has been recommended that seals needed to be protected from attacks by other seals or sympatric species whilst anaesthetised or during recovery (Gales 1989).

Desertion

Baker *et al.* (1988) found that 20% of females anaesthetised during the breeding season with ketamine and diazepam deserted their pups and many abandoned their pups briefly or showed short term confusion. These effects were not observed with the tiletamine and zolazepam combination (Baker *et al.* 1990). Bester (1988) has proposed that a rapid recovery is desirable in order to promote a rapid resumption of pup attendance by lactating cows. It is not known whether anaesthesia of mothers with pups increases desertion rate.

Escape to sea and drowning

Mitchell and Burton (1991) reported that females often attempted to reach the sea when disturbed, and could not be stopped with boards. Three escaped after receiving anaesthetic agents. Two were rescued from deep water. One was anaesthetised and subsequently died after prolonged respiratory distress. The other was only sedated and was intermittently surfacing to breath. The third seal was retrieved from shallow water before it could inhale any water. No male seals attempted to escape into the sea, and could usually be driven away from the water.

Size and the problem of the bulls

Few bull southern elephant seals have been anaesthetised (Ryding 1982, Gales and Burton 1987 a). Immobilization of these animals appears less successful than of smaller animals; four of eight bulls administered ketamine by Woods (unpublished data) died. However, whether they represent a greater risk has not been discussed.

Other problems associated with chemical restraint

Logistics

Logistically southern elephant seals can be difficult to work with. They are usually found in isolated areas where facilities are limited. Getting equipment to the areas can be difficult, often requiring long boat, walking or road trips, or the use of helicopters or amphibious vehicles. In many instances this excludes the use of sophisticated monitoring equipment or anaesthetic machines and often equipment has to be limited to that which can be easily carried. Field work limits equipment to that which is robust, battery powered, has few working parts, can be easily maintained and can be made water, cold, and shock-proof.

Working in the cold

Working in the cold can be physically demanding. The duration of surgery is constrained by the cooling of the surgeon's hands. The worker's attention span can diminish and decision making can become impaired. These difficulties can be overcome to varying degrees by acclimatisation and forethought.

At temperatures below $0^{o}C$ anaesthetic drugs can freeze or crystallize out of solution (Cline *et al.* 1969, Gales and Burton 1987 a, Baker *et al.* 1988). This problem is exacerbated by the need to use concentrated solutions of drugs in order to avoid injecting large volumes. Hand warmers or keeping the drugs held against the body until use has been used to minimise this problem (Baker *et al.* 1988).

Body mass and drug dose

i. Estimating mass of seal directly

To determine drug dose an accurate estimate of body mass is required. In most cases wild, free-ranging southern elephant seals cannot be weighed prior to drug administration and their mass must be estimated. Mass can be estimated by eye based on previous experience weighing the animals (Cline *et al.* 1969, Baker *et al.* 1988, Woods *et al.* 1989). This is difficult and can lead to incorrect dosage. Ross and Saayman (1970) reported that they had underestimated the body mass of a young male elephant seal by 50%.

ii. Estimating mass from length of seal

Length-mass relationships have been developed and used for estimating masses of southern elephant seals (Laws 1960, Vergani and Spairani 1980, Ling and Bryden 1981, Vergani 1985, Gales and Burton 1987 a). However, large fluctuations in fat content during moulting and breeding seasons cause marked mass variations leading to inaccuracies (Ryding 1982, Gales and Burton 1987 a, Woods *et al.* 1989). Therefore length-mass relationships may not be valid between animals at different times of their yearly cycle. For this reason, Gales and Burton (1987 a) recommended that workers estimating body masses of these seals for drug doses should consider the time of the animal's life cycle and time of year.

The snout tail length, a straight line measurement from the tip of the nose or proboscus to the tip of the tail, is currently used in referring to the length of animals (Laws 1960, Ling and Nicholls 1963, Ross and Saayman 1970, Morgan *et al.* 1978, Gales and Burton 1987 a, Bester 1988). Errors in measuring animal length can compound the problem of estimating mass from the length-mass relationships (Morgan *et al.* 1978). For example, Ling *et al.* (1967) found it almost impossible to judge length, and thus dose of suxamethonium, accurately enough to immobilise seals without producing apnoea.

More accurate techniques are required for estimating mass prior to drug administration in these animals.

iii. Estimating dose of drug

Difficulties in estimating drug dose arise because of both errors in estimation of mass and the variability in response to drugs (Ling *et al.* 1967, Ryding 1982). However, the uniformity of size of some southern elephant seal cohorts has encouraged the use of standard dosages as a basis for drug administration (Woods *et al.* 1989).

Doses of drugs administered to southern elephant seals have been based on those used for immobilisation of northern elephant seals, other pinnipeds or other mammals (Ross and Saayman 1970, Ryding 1982, Gales and Burton 1987 a). Once anaesthetised, more accurate dose rates have been obtained after directly measuring or weighing the animal (Morgan *et al.* 1978, Vergani 1985, Baker *et al.* 1988).

Reports of drug dosage have been based on body mass. Lean body mass has not been used for estimating drug dosage.

Variability of response

i. General

Variability in response to anaesthetic agents has been reported in the literature (Woods *et al.* 1989). Various factors such as the inability to accurately gauge body mass, the level of arousal of the animal, its sex, stage of yearly and life cycle, and the nature of drug administration, have been suggested as contributing to this variability (Cline *et al.* 1969, Bester 1988, Vergani 1985, Woods *et al.* 1989).

ii. Effect of arousal

It is well known that animals showing a period of excitement during induction of anaesthesia always require more anaesthetic (Lumb and Jones 1984, p 16) and various authors have noticed that drugs appeared to have less effect upon excited southern elephant seals. Vergani (1985) noticed that the effect of even high doses of xylazine on weaned pup southern elephant seals diminished when the injected animal became excited. Bester (1988) suggested that it was likely that response to anaesthetics varied with level of activity, stress or emotional state, the level of activity or arousal increasing tolerance. He therefore recommended that immobilizing or tranquillizing agents should preferably be delivered to undisturbed seals.

There is evidence to suggest that southern elephant seals can be stressed by administration of some immobilization drugs. "Sand-throwing", a displacement or substitution activity engendered in times of stress (Laws 1956a, Carrick *et al.* 1962), was seen by Ling *et al.* (1967) as animals were recovering from immobilization with suxamethonium. Griffith *et al.* (1979) found that immature males became very difficult to approach for injection of suxamethonium after several "bleedings" and attempts at immobilisation became less and less successful.

iii. Variability due to stage of the yearly cycle and sex

Stage of the yearly cycle appears to be an important determinant of the response to anaesthetic drugs (Cline *et al.* 1985, Woods *et al.* 1989). Duration of sedation of southern elephant seals that are pre-

moulting or in the early stages of lactation can be half that of animals which have finished moulting or lactating, despite similar dose levels of drugs (Woods *et. al* 1989).

Various authors have suggested that responses to drugs may vary with the amounts of blubber and muscle as proportions of the total body weight (Bester 1988, Woods *et al.* 1989), and with reproductive state (pregnancy) as well as the size of the animal and therefore probable differences in basal metabolic rates (Briggs *et al.* 1975, Woods *et al.* 1989). However Ryding (1982) considered that as the poorly vascularized blubber is minimally involved in the initial distribution of ketamine and hence its concentration in the blood, the fluctuation in amounts of blubber as a proportion of body weight at different times of the year may not be of great importance. Which of these factors are most responsible is unknown though Woods *et al.* (1989) did suggest that fluctuations in amounts of fat may be an important consideration and should be taken into consideration when deciding upon dosage of drug to be administered. Anaesthetic pharmacokinetic studies at different times of the animals' life cycle are indicated.

There is little information available on the effect of sex on the response of southern elephant seals to anaesthetic agents. Morgan *et al.* (1978) found that, in general, female southern elephant seals tended to be immobilised for longer than males given similar doses of phencyclidine, though it was not stated if the difference was significant. Ling *et al.* (1967), who used suxamethonium to immobilize southern elephant seals, presented results which suggested that with lower doses there were proportionally more failures to immobilize males, while at higher levels more deaths occurred among females. Whether males were more resistant to the drug could not be established with the data available.

iv. Variations in time of induction and duration

The time interval between initial drug injection and the point of immobilization can vary greatly (Cline *et al.* 1969). In one study, induction period and duration of anaesthesia were not closely correlated with drug dose (Mitchell and Burton 1991). The method of drug administration is thought to be partly responsible for this problem. When administering drug, placement of the needle is, by necessity, often hurried and the variability in response may be due to misplacing the injection outside the muscle mass (Ryding 1982) or injection into different body regions from which the rate of absorption of the drugs/ tranquillizers may vary (Bester 1988, Parry *et al.* 1981). Needles often bend, are not long enough to penetrate the blubber layer or, when attached to darts, are blown off target by wind, leading to accidental intra-fat administration (Flyger *et al.* 1965, Cline *et al.* 1969, Morgan *et al.* 1978, Vergani 1985).

Geographical location has also been implicated in variability in response to drug administration. Vergani (1985) found that estimated doses of xylazine required to obtain good immobilisation of weaned pups differed between Patagonia and Antarctica. He thought the larger estimated dose used in Antarctica may have been due to a delay in sleep induction due to the lower temperatures or to lesser precision in dart throwing caused by the strong prevailing wind. Whether the difference was significant was not stated.

Suspected intravascular administration has been implicated in differences in induction times (Gales and Burton 1987 a, Baker *et al.* 1988). In both cases effect upon the animal was more rapid than had drugs been administered intramuscularly.

Recommendations for chemical restraint and anaesthesia

Few recommendations for chemical restraint have been made for southern elephant seals. Gales (1989) however made recommendations for pinniped anaesthesia which are largely applicable, including: use of cyclohexamine drug combinations, accurate assessment of mass and physiological state of animals prior to drug administration, monitoring temperature and cooling should it rise, protection from other seals or sympatrics, use of atropine, and use of doxapram and intubation and ventilation if possible for treatment of apnoea. He concluded that tiletamine and zolazepam appeared to be the safest cyclohexamine based drug combination for use in pinnipeds, however further trials were necessary to determine the overall efficacy of the drug. He commented that combinations of ketamine and xylazine, and ketamine and diazepam, were generally recommended for use in pinnipeds.

It is currently unknown which of the commonly used anaesthetic combinations is most suitable for use in southern elephant seals. Fatality rates have been presented for some drug combinations that have recently been used; 4.5% for ketamine, 3.0 and 3.3% for ketamine and xylazine, and 1.2% for ketamine and diazepam (Mitchell and Burton 1991). The most recently used drug combination, tiletamine and zolazepam has been reported as being the most useful by Baker *et al.* (1991) who administered the drug a total of 195 times to 90 animals with no fatalities and only 6 episodes of apnoea, however (Mitchell and Burton 1990) reported 2 fatalities and respiratory problems associated with its use in 5 animals. Baker *et al.* (1991) could offer no explanation for the apparent difference seen in response. The doses of the 1:1 combination of tiletamine and zolazepam given to the two elephant seals that died in the study by Mitchell and Burton (1991) (2.4 mg/kg) were only marginally greater than the doses required for anaesthesia. Gray *et al.* (1974) obtained good immobilization of northern elephant seals with doses of 1.0 to 2.0 mg/kg (and 1.0 to 2.5 mg/kg of a 2:1 combination), Hammond and Elsner (1977) used doses of 0.5 mg/kg on southern elephant seals, and Baker *et al.* (1990) obtained good immobilization of southern elephant seals with single injections of 0.95 ± 0.22 mg/kg and multiple injections totalling 1.15 ± 0.37 mg/kg.

Based on these findings ketamine and diazepam or tiletamine and zolazepam would appear to be the most useful. However comparative studies have not been done.

Specific recommendations as to prevention and treatment of apnoea, possible associated dive-response and complications have not been made, though Gales (1989) has recommended examination of some anaesthetic reversal agents.

Chapter 3: General Materials and Methods

Selection of animals

The study was largely restricted to mature pre-moulting females, animals considered to be "low-risk" by the Ethics Committee. However, the Committee also allowed access to a limited number of mature female pre-breeding and post-moult animals specifically for pharmacokinetic work (Chapter 7). Samples were also taken opportunistically from other categories of animals (to which the Ethics Committee had allowed access) which were being restrained for other purposes including diving recorder and satellite tracking device deployment (and retrieval) and gastric lavage (Chapter 6). In all cases, the preferred subjects for trials were quiet, somnolent animals which had not previously been restrained and were located away from wallows or the sea, in positions where there was safe access and little danger to the operator or subject. Animals which were part of small groups were also preferred as they often appeared more tractable after needle placement and appeared less likely to react aggressively or move away than solitary animals. (See also Classification of animals below)

Equipment and drugs used

Sources of equipment and drugs, and specifications, used during trials are presented in Tables 3.1 and 3.2 respectively. Drugs were stored at room temperature (approximately $10^{o}C$) except for suxamethonium which was kept refrigerated at 4 to $8^{o}C$.

Drug administration

Remote injection technique

Anaesthetic agents were initially administered to southern elephant seals using a remote injection technique based on that of Ryding (1982) (Plate 3.1).

A reusable, 3.5 m length of plastic tubing (volume 27 mL) was constructed by joining approximately two and a half lengths of blood/ solution infusion set together. An 18 G, 90 mm needle was attached to one end and the plastic tubing filled through the other with saline. Part or all of the dose of drug to be administered was then introduced into the tubing. The volume of drug varied from approximately 2 to 70 mL depending upon the type of drug and the size of the animal.

(At the end of each work period the plastic tubing was washed in Hibitane Disinfectant [Chlorhexidine digluconate 8 g/dL][Coopers Animal Health, Silverwater, New South Wales, Australia], flushed, then

Table 3.1. Sources and specifications of equipment used during this study. (OD = outside diameter, FG = French gauge, ID = inside diameter.)

Item	Specifications	Supplier
0.9% Sodium chloride	Intravenous infusion BP	Baxter healthcare Toongabbie, New South Wales, Australia
Block and tackle	Anchor 1 tonne mini chain block with 3m lift	J. Blackwood and son Launceston, Tasmania, Australia
Blood/ solution infusion set	15 drops/mL	Travenol laboratories Old Toongabbie, New South Wales, Australia
Blood gas analyser	Corning 165/2 pH	Corning glass works Medfield, Massachusetts, USA
	Reagents, gases and parts	Ciba Corning diagnostics Medfield, Massachusetts, USA
Demand valve	Hudson 5090	Hudson oxygen sales Temecula, California, USA
Duodenal tube	FG 14	Indoplast Sydney, New South Wales, Australia
Endotracheal tube	Non-sterile (reusable) Aire-cuf: ID 7.0mm, OD 10.0mm ID 9.0mm, OD 12.3mm ID 11.0mm, OD 15.7mm ID 18.0mm, OD 24.0mm ID 22.0mm, OD 30.0mm ID 26.0mm, OD 35.0mm	Bivona Gary, Indiana, USA
Eye-stream	Balanced salt eye irrigating solution	Alcon laboratories Brookvale, New South Wales, Australia
Flipper tags	Jumbo Rototags	Dalton supplies Woolgoola, New South Wales, Australia
Hibitane disinfectant	Chlorhexidine digluconate 8g/L	Coopers animal health Silverwater, New South Wales, Australia
Hypodermic needle	18Gx1 1/2", 1.25x38mm 25Gx3/4", 0.5x16mm 14Gx8"	Terumo Tokyo, Japan The amalgamated dental Australia Carlton, Sydney, Australia
Obstetrical lubricant		Parnell laboratories Silverwater, New South Wales, Australia
Oesophageal stethoscope	120cm	Portex, Hythe, Kent, England
Oxygen cylinder	C, D, E, G size	CIG Medishield Hobart, Tasmania, Australia
Regulator	C size D/E/G size	CIG Medishield Hobart, Tasmania, Australia
Spinal needle	18Gx3 1/2", 1.20x90mm	Terumo Tokyo, Japan
	19Gx8.89cm	Becton-Dickinson Franklin Lakes, New Jersey, USA
Surgical catgut, BP	Chromic, Metric 3.5 (2/0) eyeless needle sutures cutting 75cm	Ethnor Sydney, New South Wales, Australia
Suture needle	Size 12, half circle cutting	P. Spencer and sons Redditch, England
Syringe	1mL, 2mL, 5mL,10mL,20mL,50mL	Terumo Melbourne, Australia
Weighing scales	Challenger 2.5 ton digital scale	
Weighing slings		Hood sails Hobart, Tasmania, Australia

Table 3.2. Sources of drugs, specifications and solutions for injection of drugs used during this study.

Nonproprietary and generic name	Preparation	Supplier
4-aminopyridine (Xylex injection)	24 mg/mL	Parnell Laboratories Caringbah, New South Wales, Australia
Adrenaline tartrate (Adrenaline tartrate injection)	1 mg/mL	Parke Davis Caringbah, New South Wales, Australia
Atipamezole hydrochloride (Antisedan vet. inject.)	5 mg/mL	Farmos Group Turku, Finland
Atropine sulphate (Atrosine mitis)	0.65 mg/mL	Parnell Laboratories Caringbah, New South Wales, Australia
Diazepam (Valium)	10 mg/2mL	Roche Products Dee Why, New South Wales, Australia
Doxapram hydrochloride (Dopram-V)	20 mg/mL	Bomac Laboratories Castle Hill, New South Wales, Australia
Fentanyl citrate USP (Fentanyl Citrate Injection USP)	50 ug/mL	David Bull Laboratories North Clayton, Victoria, Australia
Flumazenil (Anexate)	1 mg/10mL For clinical trial use only	Roche Products Dee Why, New South Wales, Australia
Heparin (Heparin Injection B. P. Contains no antiseptic)	5000 U/0.5mL	Commonwealth serum laboratories Melbourne, Victoria, Australia
Heparin (Heparin Injection B. P. mucus)	5000 U/0.5mL	Commonwealth serum laboratories Melbourne, Victoria, Australia
Ketamine hydrochloride	186 mg/mL (160 mg/mL ketamine base)	Parke Davis Caringbah, New South Wales, Australia
Ketamine hydrochloride (Ketalar)	100 mg/mL ketamine base	Parke Davis Caringbah, New South Wales, Australia
Medetomidine (Medetomidin vet. inj.)	10 mg/mL For investigational veterinary use	Farmos Group Turku, Finland
Midazolam hydrochloride (Hypnovel)	5 mg/mL	Roche Products Dee Why, New South Wales, Australia
Naltrexone HCL	1 mg/mL	Sigma Pharmaceuticals Clayton, Victoria, Australia
Naloxone hydrochloride (Naloxone HCl injection USP)	0.4 mg/mL	David Bull Laboratories Mulgrave, Victoria, Australia
Pentobarbitone sodium (Apex Euthanasia Solution)	400 mg/mL	Apex Laboratories St. Marys, New South Wales, Australia
Pethidine hydrochloride (Pethidine)	50 mg/mL	Parnell Laboratories Caringbah, New South Wales, Australia
Sarmazenil	Powder (made up to 2.5 mg/mL)	Roche Products Dee Why, New South Wales, Australia
Sodium bicarbonate 8.4% w/v, injection, B.P.	4.2 g sodium bicarbonate 1000 mmol sodium and bicarbonate ions/ L	International medication systems So. El Monte, CA 91733, USA.
Suxamethonium chloride B. P. (Scoline)	50 mg/mL	Glaxo Australia Boronia, Victoria, Australia
Thiopentone sodium powder (Pentothal)	5 g (made up to 50 mg/mL)	Boehringer Ingelheim Artarmon, New South Wales, Australia
Tiletamine-zolazepam (Zoletil 100) Water for injections	250 mg base of each drug (made up to 100 mg/mL)	Virbac Peakhurst, New South Wales, Australia Abbott Australasia Sydney, New South Wales, Australia
Xylazine hydrochloride (Rompun)	100 mg/mL xylazine base	Bayer Australia Artarmon, New South Wales, Australia
Yohimbine hydrochloride (Reverzine Injection)	10 mg/mL	Parnell Laboratories Caringbah, New South Wales, Australia

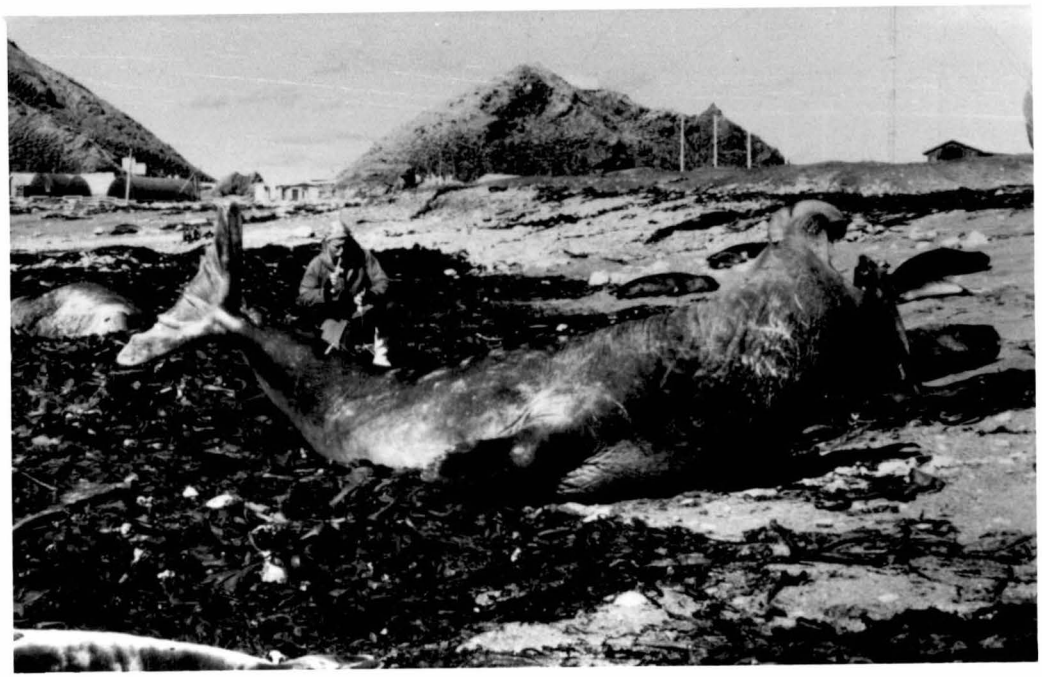

Plate 3.1. The remote injection technique used in this study (after Ryding 1982). The needle has just been inserted, the operator has moved to the end of the length of tubing and is in the process of removing the cover of the needle on the syringe of saline held in his right hand. The end of the tube is held in his left hand. Note the needle positioned in the caudal gluteal area, length of tubing and response of the bull.

heavily sedated, immobilized or anaesthetised its head response (Chapter 4) was assessed to confirm the degree of chemical restraint and work commenced with the animal.

Intravenous access could usually be assured within 10 min of using cyclohexamine drug combinations. Using narcotic combinations, or when immobilizing bulls, 15 to 20 min was allowed for onset of effect before the animal was approached and assessed. There was concern that if the animal was approached prematurely it might become frightened, preventing induction of adequate chemical restraint or possibly predisposing to apnoea (Backhouse 1964).

If at this stage the animal was still only moderately sedated an attempt was made to place a spinal needle into the extradural intravertebral vein for an additional drug dose. The needle position was chosen based on estimations of the animal's level of sedation, behaviour and location of the vein (as palpation for landmarks was often dangerous or would further disturb the seal). If the animal was close to heavy sedation (level 3; Chapter 4), intravenous access could usually be accomplished. Otherwise intravenous access could not be assured and further drug was administered intramuscularly. The procedure was abandoned in cases where the animal was considered to be excessively stressed, or behaving abnormally, or was in danger of going to sea, becoming trapped in a wallow or puddle with its face under mud or water, or in a head down or dorsally recumbent position.

Intravenous drug administration

i. General

In most instances after intramuscular drug administration, if further drug was needed it was administered intravenously into the extradural intravertebral vein which was the preferred sight for venipuncture (Plate 3.2 and Figure 3.1). The functional and anatomical features of this vessel have been described for other phocid species (Harrison and Tomlinson 1958, Hol *et al.* 1972, St. Pierre 1974, Ronald *et al.* 1974). The plantar venous arch (St. Pierre 1974) was used when extradural intravertebral vein access could not be achieved (Plate 3.3 and Figure 3.2).

Speed of injection varied. Drugs were usually given slowly, but they were administered rapidly if the animal was becoming fractious and the operator was in danger of being bitten. Rapid administration was generally avoided because of the risk of apnoea.

ii. Skin preparation

Sites for venipuncture in southern elephant seals were often found to be contaminated with faeces, wallow water or mud. Lavaging with copious buckets of sea water and a scrubbing brush was found to be more effective at removing surface contamination than use of small volumes of disinfectant. For this reason sea water and not disinfectant was generally used to prepare the skin prior to

filled with saline. At the beginning of the next work period the tube was flushed with fresh saline prior to introducing drug.)

The needle was inserted tangentially into the lumbar or gluteal muscle mass of the animal (Bryden 1971). If necessary an assistant stood in front of the animal to distract its attention and allow a second person to insert the needle and administer the drugs.

After checking to ensure that the needle was not intravascular by withdrawing on the plunger of the syringe, the drugs were administered intramuscularly through the tube and flushed through with saline, using a volume larger than the volume of the tube, usually 40 mL. After injecting each 10 mL of saline, a check was made for intravascular administration by withdrawing on the plunger of the syringe. If blood flashed back into the hub of the needle or tube, or if the force required to inject the drugs was less than usual, administration of drug was stopped and the animal's response observed. If intravascular administration was suspected the needle was withdrawn slightly, redirected or reinserted. It was found that slight withdrawal usually caused the least disturbance to the animal. The needle was left inserted in case further drug had to be administered intramuscularly. Using this technique, time for injection of drug and flush varied from 10 s to 2 min depending on the volume delivered and response of the animal.

If possible, the operators then retreated to a position which was out of direct sight, scent and sound of the animal but from which the animal's breathing could be observed. In this way the animal was left alone for 10 min as the drugs began to take effect. Breathing, as witnessed by opening and closing of the external nares, chest movement or sounds of exhalation, was surreptitiously checked every few min without entering the animal's flight distance. If the animal stopped breathing within this 10 min period, or appeared to be excessively deeply anaesthetised, it was approached and the head or tail stimulated, the animal rolled over, or a needle inserted into the extradural intravertebral vein (for administration of a drug or for blood sampling) to stimulate breathing.

At the end of the 10 min period, or if the animal appeared anaesthetised before this time, it was slowly and cautiously approached by one person. By walking slowly past along a semicircular route the operator would enter the animal's flight distance near its head, observing its response. If the animal reared up or attempted to move away, the operator retreated and left the animal for a further 10 min. If the animal did not rear up or move away, it was approached to within a few feet of its head. If the animal still failed to rear up or move away its caudal flipper response (Chapter 4) was checked. The cranial end of the animal was then approached by running a hand along its body in a caudal to cranial direction. This approach, rather than walking up and touching the head, was used as it allowed the operator to further assess the response of the animal to touch whilst staying out of bite range. Moving further cranially the withdrawal response (Chapter 4) was tested. If the animal was considered

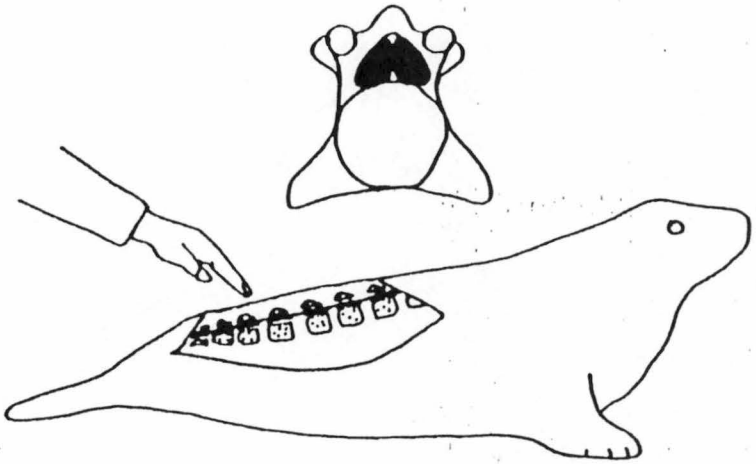

Figure 3.1. (above). Schematic diagrams of the location of the extradural intravertebral vein in a weaner southern elephant seal including a cut away median section after Geraci and Sweeney (1978) and a cross section of a lumbar vertebrae from a mature bull. The hand shows the approximate location for venipuncture (see also **Plate 3.2**, left).

Plate 3.2. (left). Venipuncture of the extradural intravertebral vein of an anaesthetised weaner.

Plate 3.3. Venipuncture of the plantar venous arch of a bull. The left caudal fluke has been rotated outward so that the plantar surface is exposed. The skin is being prepared with alcohol and hibitane (operator's right hand). A 20mL syringe with an 18G, 38mm needle is held in the operator's left hand.

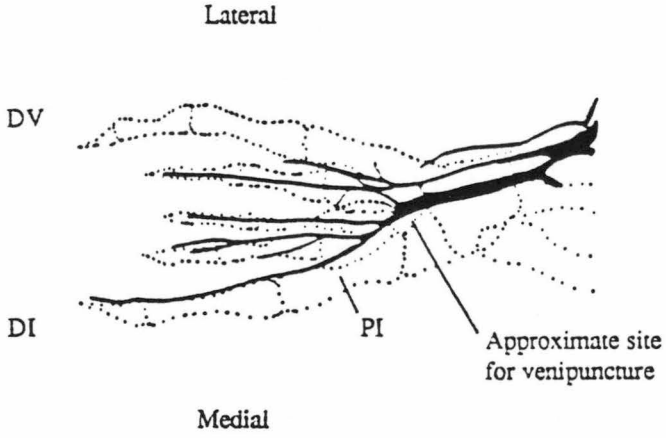

Figure 3.2. Schematic diagram of the location for venipuncture of the left plantar venous arch (plantar view after St. Pierre 1974). The needle is introduced in a cranial direction, parallel to the fluke of DI (medial to PI) at an angle of about 45° to the skin at the junction of the interdigital webbing, or just cranial to this point.

venipuncture. An attempt was made to always introduce needles through relatively uncontaminated skin.

Three steps were used to disinfect the skin at the surgical site prior to surgery. Gross surface contamination was removed by scrubbing and lavage with copious amounts of sea water. The skin was then scrubbed with an 8 g/L solution of chlorhexidine digluconate. Finally, just prior to making the initial incision, the skin was wiped with a solution of chlorhexidine digluconate in alcohol (previously prepared by adding 100 mL of 8 g/L solution of chlorhexidine digluconate to 900 mL 70% alcohol).

iii. Venipuncture of the extradural intravertebral vein

Positioning of animals for venipuncture - Venipuncture of the extradural intravertebral vein was most easily achieved with the subject in sternal recumbency, in a horizontal plane, with the spine slightly flexed. Venipuncture could be achieved in almost any position except dorsal recumbency. However, in all positions other than sternal recumbency the fat and muscle layers moved to varying degrees with respect to the underlying bony structures, which made location of land marks more difficult. Extension of the spine made palpation (and insertion of the needle) between the vertebrae more difficult as the diameter of the spaces between them decreased with increased extension of the spine.

If the animals could not be positioned in a horizontal plane they were positioned with the head up-hill.

Landmarks for venipuncture - The dorsal midline, wings of the ilium and intervertebral spaces were used as landmarks to locate the extradural intravertebral vein. Landmarks were most easily palpated in thin animals after the breeding season or moult. Often in fat animals and bulls (and animals insufficiently sedated) no landmarks could be palpated. In these animals the positioning of the animal and the location of the black line of hairs down the back were used as guides to the location of the vessel.

The dorsal midline was located by palpating the wings of the ilium and bisection of the distance between them. There was often a narrow line of black hair running along the dorsal midline of the animal which was used as an aid in locating the dorsal midline. The craniodorsal aspect of the base of the sacrum was palpated using firm digital pressure along the dorsal midline between the wings of the ilium. The space between any two vertebral bodies in the caudal lumbar area was used for venipuncture, however the preferred sight for placement of needles for drug administration and sampling was approximately 30 to 45 cm forward of the proximal end of the sacrum (5 or 6 vertebral bodies in a mature female). On dissection of naturally deceased mature pre-moulting female animals it was found that at this level the extradural intravertebral vein was paired and approximately 4.5 cm in total width.

Blood flow in this vessel has not been described in the southern elephant seal but has been described in another phocid, the harp seal (Hol *et al.* 1972, Ronald *et al.* 1974). In the resting, nonbradycardic seal, dye injected into the extradural intravertebral vein at the thoracic, lumbar or sacral level flowed anteriorly in the extradural intravertebral vein and returned to the heart via the thoracic intervertebral veins, or the cervical vertebral venous sytem. However, in the diving or bradycardic seal, dye flow was reversed and the cerebral blood (which was presumed not to be diluted with blood from other tissues), flowed along the length of the extradural intravertebral vein, and passed both into the anterior, and the posterior vena cava. The resting seal often exhibited a brief bradycardia in association with apnoea in which the flow patterns observed closely resembled those of diving. The change from a resting to diving pattern of blood flow occurred when the heart rate fell to 40 to 50 bpm (Ronald *et al.* 1974).

Acid-base and blood gas changes were measured in this vessel. To minimise any direct effect, such as changing pH, that further drugs administered in to this vessel might have on these variables, needles for drug administration needed to be placed so that they were down stream of those for blood sampling. This created conflict in location of catheters due to the potential changes in direction of flow in this vessel. However, as most animals breathed regularly during chemical restraint it was presumed that blood flowed anteriorly in this vessel and drug administration needles were located approximately 1 to 2 intervertebral spaces cranial to the blood sampling needles. For trials where drugs were administered to apnoeic animals by this route, drug administration needles were located approximately 1 to 2 intervertebral spaces caudal to the blood sampling needle. Changes in flow in this vessel were taken into account when interpreting acid-base or blood gas values recorded post additional drug administration (See: Interpretation of blood gas values below).

Dissection of the lumbar area of dead seals revealed that needles had to be passed between the dorsal laminae of two vertebrae. At this level, spinous processes were small, and the land marks being palpated to locate the "intervertebral space" were the concave, cranial edge of the dorsal lamina running between the articular processes of the more caudal vertebra and the concave, caudal edge of the dorsal lamina running between the caudal articular processes of the more cranial vertebra. A slightly flexed spine was found to facilitate palpation, location and placement of needles as the "intervertebral spaces" were "opened up". Extension, or straightening, of the spine caused them to "close", preventing entry of the needle when the spine was overextended.

Technique - With the probing finger as a guide, an 18 or 19 G, 90 mm needle was inserted with the stylet removed, perpendicular to the skin of the dorsal midline, between 2 vertebral bodies and pushed down into the extradural intravertebral vein. Often penetration of the ligamentum flavum was felt and blood flashed back into the hub of the needle. In some instances air was heard being drawn into the

vein as it was entered or flashing back of blood did not occur* . If so, the hub of the needle was immediately capped with a 1 mL syringe, which was then used to test for intravenous access (Plate 3.2 and Figure 3.1).

In some cases, despite indications of intravenous access, blood sampling could not be performed, presumably because the bevel of needle was blocked by contact with the vessel wall. The needle was then rotated, to unblock the needle and allow blood to be taken. It was found that in some animals venous pressure was such that 1 mL sampling syringes filled automatically. When this happened the 1 mL syringe was replaced with a 20 mL syringe; the greater resistance of its plunger preventing filling.

Needles used - Eighteen G, 90 mm needles were preferred as blood seemed to flow more freely from them and they appeared to become blocked less often than 19 G, 90 mm needles.

Eighteen G, 90 mm needles were usually, but not always, long enough to allow venipuncture of mature cows in good body condition. In most females in good condition the length of the needle placed the tip within the lumen of the extradural intravertebral vein when the luer connection was in contact with the skin. In some larger females, the hub of the needle had to be pushed down where it came in contact with the skin, which created a trough in the skin, up to 3 cm deep, to allow the vessel to be reached. In thinner animals after moult or parturition care had to be taken not to punch through the base of the vein. On dissection of naturally deceased animals it was found that the spinal cord had finished at this level, although four large nerve bundles ran along the base of the spinal canal. Thus in thin animals (and pups) needles were introduced cautiously, or with applied suction to prevent potential nerve damage from passing the needle through the vein. Excess probing in the area was avoided.

Pups and weaners were bled from the extradural intravertebral vein. Depending upon size; pups were usually bled using an 18 G, 38 mm needle, and weaners generally required an 18 G, 90 mm needle. It was found to be important to keep needles patent by use of a stylet or anticoagulant (20 IU/ mL heparin).

iv. Plantar venipuncture; the "tail vein"

In some bulls and cows, the extradural intravertebral vein could not be reached with an 18 G, 90 mm needle due to the animal's size or fat coverage. In these animals venipuncture was achieved using the plantar venous arch (arcus venosus plantaris) or "tail vein" (St. Pierre 1974).

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* These observations implied wide variability of various pressure within this vessel even in the normal resting, non-apnoeic state.

The anatomy of the veins of the caudal flipper has not been described in elephant seals. On dissection of the caudal flipper of naturally deceased animals it was found that the vasculature appeared to have similarities to that seen in the harp seal (*Pagophilus groenlandicus*; St. Pierre 1974). In bulls the vein was up to 3 cm deep. It ran on the plantar surface of the flipper, along the medial margin of DI, coalescing with other vessels from the dorsal aspect at the junction of the interdigital webbing. With the left caudal flipper rotated supine, DV was abducted to form an angle of approximately 70 to 90^{o} with DI, venipuncture was achieved with a 38 mm, 18 G needle introduced in a cranial direction, parallel to the fluke of DI (medial to PI) at an angle of about 45^{o} to the skin at the junction of the interdigital webbing, or just cranial to this point at a spot that can be recognised, on palpation, by the proximal portion of the metatarsals which form a relatively immobile group of bones (Plate 3.3 and Figure 3.2).

Further anaesthetic was also administered to one yearling, whose tail and lumbar area were contaminated with faeces, using a vein that was found to run obliquely across the palmar surface of the left fore flipper (palmar venous arch; St. Pierre 1974). This vessel needed to be occluded to allow venipuncture. The "tail vein" did not need to be occluded.

Endotracheal drug administration

The animal was intubated with an endotracheal tube, and a sterile, duodenal tube with the proximal end cut off was passed down the lumen of the endotracheal tube. The duodenal tube was inserted approximately 5 to 10 cm past the distal end of the endotracheal tube. A syringe, with luer fitting, containing doxapram or saline was attached to the proximal end of the duodenal tube and the contents administered rapidly. The syringe was removed and the duodenal tube flushed with 50 mL of air. The duodenal tube was then removed.

Intralingual drug administration

Injections were made intramuscularly into the body of the tongue, by inserting an 18 G, 38 mm needle through the lateral surface into the genioglossus muscle (Bryden 1971 b, Evans and DeLahunta 1980). Ten mL of fluid was the maximum administered in any one spot before the needle was removed and relocated. No attempt was made to test for intravascular administration.

Blood gas analysis

Blood gas analysis was performed using a Corning 165/2 pH Blood Gas Analyser. All gases and reagents used in maintenance and operating procedures were manufactured by, and purchased from, Ciba Corning Diagnostics Corporation, Medfield, Massachusetts, USA.

Preparation and calibration of analyser

Daily preparation and calibration procedures were performed according to the Corning Medical 165/2 pH Blood Gas Analyser Instruction Manual. Each morning and afternoon a complete calibration and slope procedure was carried out. Before each series of samples were run a one point calibration check was performed.

To calculate calibration values for gases the ambient barometric pressure was recorded in millibars (mb) before each pH and gas electrode calibration. The value was corrected and reduced to station level pressure by the bureau of meteorology on the island. Pressure in mb was converted to mmHg using the relationship:

1 mb = 0.7501 mmHg (Halliday and Resnick 1962).

The water vapour pressure at the humidifying temperature of 37°C (47 mmHg) was then subtracted and this value used to find the correct calibration points for CAL and SLOPE gases from a blood gas barometric pressure chart supplied by the manufacturer. Gas calibration standards consisted of CAL GAS (12% O_2, 5% CO_2, balance N_2) and SLOPE GAS (10% CO_2, balance N_2).

Two commercially prepared buffer solutions were used in the calibration of the pH electrode (Ciba Corning Diagnostics, Medfield, Massachusetts, USA). Blood Buffer pH 6.838 contained: disodium hydrogen phosphate, 0.025 molar; potassium dihydrogen phosphate, 0.025 molar; and a preservative. Blood Buffer pH 7.382 contained: disodium hydrogen phosphate, 0.0085 molar; potassium dihydrogen phosphate, 0.034 molar; and a preservative.

Quality control was carried out after one point calibration each morning using a commercially prepared buffered electrolyte solution (Certain + Level 1 pH/ Blood Gas and ISE Quality Control; Ciba Corning Diagnostics, Medfield, Massachusetts, USA) that had been equilibrated prior to packing to yield expected values for pH, P_{CO2} and P_{O2} in properly functioning analyzers.

Preparation of blood gas collection syringes

Syringes were heparinised with 0.4 mL of a 20 IU/ mL heparin sodium solution (1 mL Heparin Injection B.P. 5000 Units in 0.5 mL, added to 500 mL saline). Approximately 0.4 mL of this solution was drawn into each plastic 1 mL tuberculin syringe, a 25 G needle applied, air bubbles flicked out of the syringe, and the syringe stored at approximately 10°C until required for use.

Use of concentrations of heparin of greater than 1000 Units/ mL tend to increase P_{CO2} and reduce pH (Adams and Hahn 1979). The 20 IU/ mL solution used during trials was thus considered to have negligible effect on these variables. The dilution effect of the heparin solution was unknown. In humans the dilution effect of the sample by heparin, causes a fall in P_{CO2} and HCO_3^-, but is

generally ignored for clinical purposes (Adams and Hahn 1979). As sample volume, and volume and concentration of heparin were constant, dilution effects were ignored whilst analysing blood gas data during this study.

Venous blood collection

It had been hoped that arterial blood could be collected. However, this was found to be impractical considering the arterio-venous anastomoses and deep location of vessels, the sampling protocol, depth of anaesthesia and use of short acting, injectable anaesthetic agents. For these reasons samples for analysis were collected from the extradural intravertebral vein of seals that had previously been sedated or immobilised with anaesthetic agents.

Prior to anaesthesia for blood gas blood collection, the heparinised 1 mL syringes were labelled 1 to 10 using an indelible ink pen (if further samples were required labelling occurred in the field). An 18 G, 90 mm needle was placed within the extradural intravertebral vein of an immobilized animal, and the first 1 mL syringe attached to the luer fitting of the needle, the heparin solution contained within it injected, and 1 mL of blood slowly aspirated. The 25 G needle was replaced, the syringe checked for air bubbles which, though rarely seen, were removed if necessary, the needle crimped, and the syringe placed in an ice water bath until assayed (usually within 30 to 60 min).

The next heparinised syringe was immediately attached to the spinal needle and approximately 0.2 mL of the heparin solution within it used to flush the spinal needle. Five min later the final 0.2 mL of heparin solution remaining within the syringe was injected and 1 mL of blood aspirated as above. If possible this procedure was repeated at 5 min intervals for the first 50 min of the anaesthetic episode. In many cases sampling had to be terminated before the desired 50 min as animals woke up and sampling became dangerous. If anaesthesia was more prolonged the frequency of sampling was reduced to every 10 min.

Assay of samples

On return to the laboratory each syringe was removed from the ice-water bath, rolled between the palms of the hands for several seconds then analysed on the blood gas analyser at 37^{o}C using the technique outlined in the manual. After equilibration for 2 min the pH, Pv_{CO2}, Pv_{O2}, HCO_3^-, total CO_2, and base excess were recorded. Pv_{CO2} and Pv_{O2} were measured in mmHg; HCO_3^-, total CO_2 and base excess in mmol/L. Base excess was calculated from the formula described by Siggaard-Andersen (1966):

$$\text{Base excess} = (1 - 0.014\ Hb)\ [(HCO_3^- - 24) + (9.5 + 1.63\ Hb)\ (pH - 7.4)]$$

where Hb represents the patient's haemoglobin value in g/dL. A mean value for the species of 23.3 g/dL (Lane *et al.* 1972) was used in this study.

Interpretation of blood gas values

There are difficulties associated with interpretation of venous blood gas values (Haskins 1977). Central venous and capillary blood do provide approximate screening values for arterial blood gas data but are subject to considerable error in some disease conditions. Central venous blood also does not provide any information regarding the ability of the lungs to oxygenate blood but may provide reliable values for base deficit. It may provide a better estimate of the status of the interstitial fluids of the tissues than will the arterial blood but will yield misleading information in abnormalities associated with decreased tissue perfusion (Haskins 1977). Substitution of venous for arterial blood might be appropriate if the relationship between the two is constant, however because this relationship has not been determined in southern elephant seals, and because of circulatory changes during apnoea, blood gas values presented in this study need to be interpreted with caution. Furthermore, "abnormalities" in other species may not be such in seals due to their adaptations to diving. Venous blood gas values do however present useful information which is easily and quickly gathered in situations where arterial sampling is impractical.

An interesting discussion of the changes in blood flow associated with apnoea in the harp seal (another phocid seal) has been presented by Ronald *et al.* (1977). Angiography studies revealed that blood flow in the extradural vein of seals not undergoing bradycardia flowed anteriorly whilst flow became posterior with bradycardia (see Figures 9 and 10 in Ronald *et al.* 1977). Furthermore, there appeared to be some control of the ratio of oxygenated blood to deoxygenated blood entering the heart. In one case, early in the dive, two heart beats were observed without the emission of boluses of blood from the caval sphincter. This would have the effect of reducing the proportion of relatively well oxygenated, venous, hepatic sinus blood sent to the brain. If these changes occur in bradycardic, apnoeic, anaesthetised seals then interpretation of blood gas values will be difficult to impossible without knowledge of flow directions and magnitudes. Blood gas and pH values in the breathing seals in this study were taken to give a crude reflection of central arterial values for comparative purposes. However, for the previously mentioned reasons, less confidence was placed in values determined in apnoeic, bradycardic seals. Despite the difficulties with interpretation, the values determined in these animals were considered to give a crude estimate of the direction of changes in CNS arterial values.

Intubation and mechanical ventilation

Intubation technique

i. General

The anatomy of the upper respiratory tract of elephant seals has not been described. Intubation was performed blind.

To hold the mouth open in cows, two 2.5 cm x 110 cm rock climbing tapes were passed around the animal's upper and lower jaws immediately behind the canines. The tape for the lower jaws had a 15 cm diameter loop at each end so that a gumboot could be placed through it. An assistant stood, with a gumboot either through each loop or on top of the lower tape (holding the lower jaw down, or open), and applied upward force to the tape around the upper jaw (Figure 3.3 and Plate 3.4). This opened the jaws and gave the operator some protection against being bitten. Two people were required to hold open the mouth of a bull. Each stood at either side of the mouth on the lower tape and hauled up on the upper tape.

A thin layer of obstetric lubricant was applied to the caudal third of the endotracheal tube. The left hand was introduced into the mouth cavity proper with the hand prone and moved from side to side as it was advanced towards the pharynx, feeling for the cranial end of the larynx. At the root of the tongue resistance was generally encountered from the soft palate which, along with the root of the tongue and "palatoglossal arch" appeared to form a "sphincter" separating the mouth cavity proper from the pharynx in some animals. Downward pressure was put on the tongue and the hand levered under the soft palate. Once into the pharynx, pedunculated masses, presumed to be palatine tonsils, could be felt on either side of the pharynx. The cranial end of the larynx was felt on the floor of the pharynx as a hard, dorsoventrally elongated cartilagenous "button", approximately 4 cm x 3 cm in a mature cow, with the epiglottis covering it. The epiglottis was levered down and the two arytenoids and the slit between them palpated. Usually the mouth of the larynx was tightly closed but appeared to become more relaxed as level of chemical restraint increased. A flaccid larynx was associated with deep anaesthesia. Once the larynx was located the appropriate size endotracheal tube was introduced along the underside of the forearm into a funnel formed by the fingers of the left hand. Whilst holding the epiglottis down, a forefinger was forced between the arytenoids to open them slightly, a second finger inserted and the bevel of the endotracheal tube threaded into the larynx (Figure 3.3). This often required considerable force due to the strength with which the arytenoids were held adducted.

When the arytenoids were separated the animal would often either attempt to shake its head, bite down on the operator's arm or vomit. It was found that lightly immobilized (level 4 chemical restraint; see Chapter 4) pre-moulting cows would move their heads from side to side whilst biting, while heavily immobilized animals (level 5) would bite down. Generally this was not too much of a problem, other than causing some discomfort, and could be overcome to some extent by the person holding the tapes controlling the head. However, in larger animals and some cows which reacted excessively to the procedure intubation was found to be safe only when the animal was heavily immobilized or lightly anaesthetised (level 5 or 6) with a cyclohexamine. With narcotic and barbiturate anaesthesia the larynx appeared more relaxed and in some cases intubation was accomplished with little difficulty when animals were lightly immobilized.

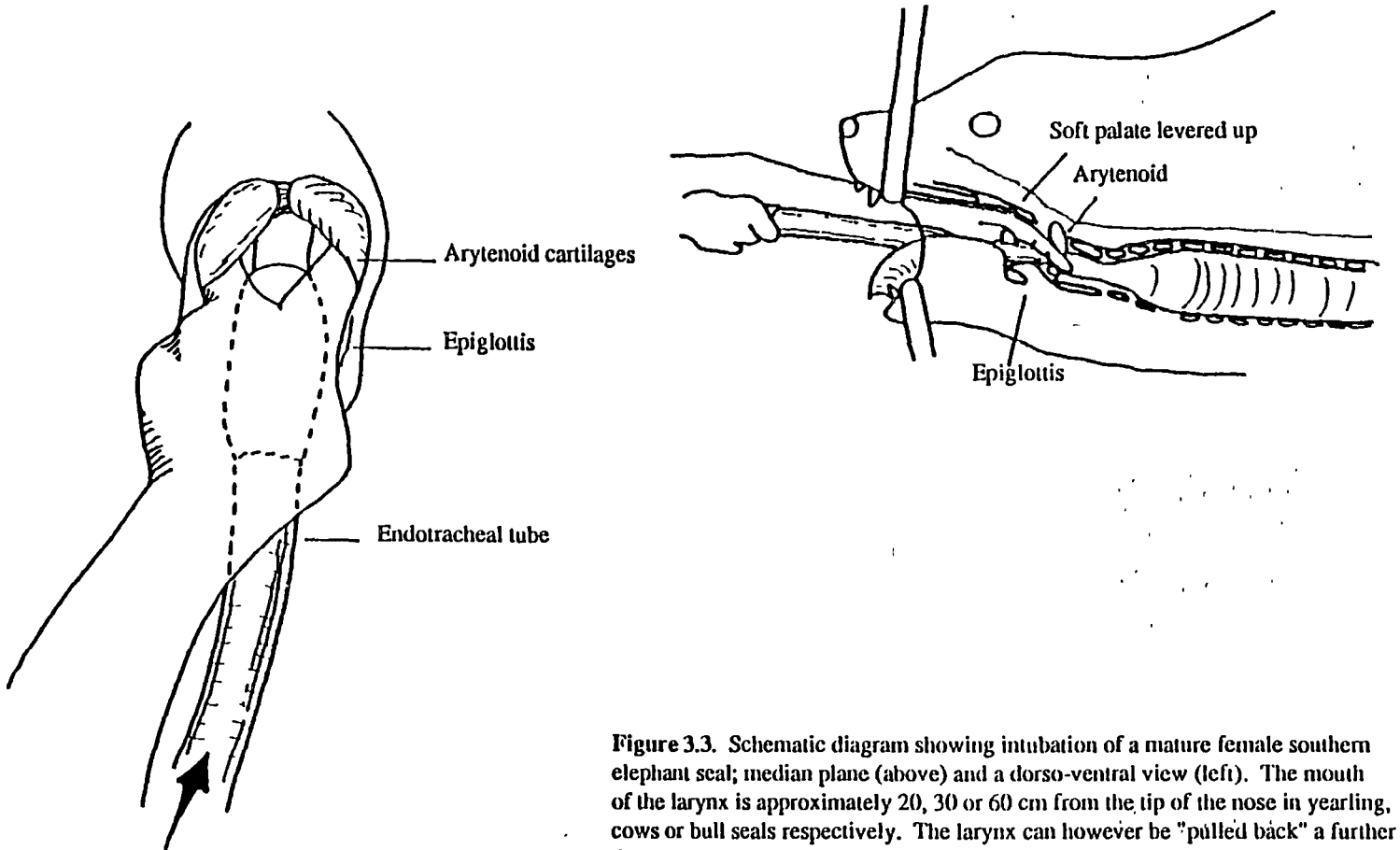

Figure 3.3. Schematic diagram showing intubation of a mature female southern elephant seal; median plane (above) and a dorso-ventral view (left). The mouth of the larynx is approximately 20, 30 or 60 cm from the tip of the nose in yearling, cows or bull seals respectively. The larynx can however be "pulled back" a further 5 to 10 cm by the animal at low levels of chemical restraint in response to touching it.

Plate 3.4. Intubation of a mature female southern elephant seal. Note the position of the tapes behind the upper and lower canines to restrain the head and hold the jaws open. The assistant is standing on the lower tape.

During monitoring bulls were found to respond more slowly or sluggishly to stimuli used to assess level of chemical restraint than cows. This can be misinterpreted as indicating an adequate level of restraint for intubation. This should be considered prior to the decision whether to intubate.

Gloves were not used as it was found that whilst inside the larynx passing the tube they tended to become tangled with the bevel of the endotracheal tube. Mouth gags also tended to get in the way.

Endotracheal tubes were kept clean by tying within a veterinary rectal glove until required.

ii. Positioning for intubation

Intubation could be performed with the animal in almost any position. However, for a right handed operator it was found to be easiest to use the left hand to open the arytenoids and the right to feed the endotracheal tube in with the animal in ventral or right oblique recumbency with the neck extended. Intubation could still be performed with the neck flexed, however this was far more difficult as the endotracheal tube had to be passed around a bend made by the mouth cavity proper and the larynx.

iii. Endotracheal tubes used

Juvenile animals (approximately 1.65 m snout-tail length) were intubated with 10.0, 12.3 or 15.7 mm outside diameter endotracheal tube, inflated or not depending upon their fit; mature cows (approximately 2.4 m snout-tail length) and young males with 24.0 mm or 30.0 mm outside diameter endotracheal tube, not inflated; and bachelor and breeding males with 30.0 mm outside diameter or 35.0 mm outside diameter endotracheal tube, inflated or not inflated depending upon fit. When a larger tube could not be passed due to tight arytenoid adduction in cows, 15.7 mm outside diameter or 20.0 mm outside diameter endotracheal tube were used.

The force with which the arytenoids were held adducted in some bulls made us suspect that the commonly used silicon or rubber endotracheal tubes might collapse and become occluded in some cases, however this was not seen. A more rigid tube, perhaps made from polyvinylchloride, might be necessary in some cases, though care would need to be taken not to damage the lining of the respiratory tract.

Prior to trials involving intubation the mouth and upper respiratory tract of a dead juvenile, mature bull and cow were dissected to familiarize the operator with upper respiratory tract anatomy. Measurements were made of the diameter of the laryngeal opening, body of the larynx and trachea, and endotracheal tube passed to assess sizes of endotracheal tube that might be required in the live animal.

Mechanical ventilation technique

Once intubated, a demand valve was attached to the endotracheal tube and the animals ventilated (see Riebold *et al.* 1980). The demand valve was attached to a C size medical grade oxygen cylinder with C size medical oxygen regulator (Plate 3.5). The lever of the demand valve was depressed for inspiration. There was concern that the valve might impede expiration of air so it was removed from the endotracheal tube for expiration. This allowed unrestricted expiration and allowed the operator to assess attempts of the animal to inspire voluntarily by inserting the forefinger into the endotracheal tube to feel for passage of air. In cows the inspiratory phase usually lasted as long as it took to achieve full chest inflation (usually about 2s and no longer than 4s), and the expiratory phase as long as expiration (usually no longer than 3s). The inspiratory: expiratory ratio was thus usually about 1:1. Attempts were made to shorten the inspiratory phase, but gas flow rates and driving pressure made this difficult. Frequency of mechanical ventilation varied depending upon mucous membrane colour and level of chemical restraint. Frequency was rapid initially in cyanotic cows until mucous membrane colour returned to normal (about 15 inflations/ min). When mucous membrane colour returned to normal, the frequency was decreased to a maintenance level (about 6 inflations/ min). To aid return to spontaneous ventilation, as level of chemical restraint decreased (< level 6) ventilation rate was decreased to 2 - 3 inflations/ min, the time between breaths increasing as level of chemical restraint decreased. Periods of several min were occasionally allowed in light animals (< level 6 restraint) to let Pa_{CO2} increase. In animals at levels of restraint ≥ 6, administration of doxapram (2 mg/kg) endotracheally, was often used to stimulate spontaneous ventilation. If spontaneous ventilation returned, assisted ventilation continued until approximately level 4 restraint when the endotracheal tube was removed. If the animal failed to to initiate spontaneous respiration and was at a low level of chemical restraint (< 6) with impending return ie twitching or opening and closing of the nostrils with no inspiration, doxapram was administered endotracheally and the endotracheal tube removed. Spontaneous ventilation usually returned within min of removing the endotracheal tube. If it did not, in most cases tapping of the face or rolling the animal stimulated breathing.

The C size cyclinder was rapidly depleted (usually in < 5 min). Its main use was for administration of oxygen whilst a larger cylinder (D, E or G) was collected from the ANARE base. G size cylinders were generally impractical due to their weight and difficulty in transporting. D size were more transportable than E size, however they did not last as long. For this reason E size cyclinders were most commonly used and lasted from 7-20 min depending upon the animal's size and frequency of ventilation.

It was found that animals < 1000 kg could be ventilated in most positions, however sternal recumbancy or with the animal on its back and side (Hammond and Elsner 1977) on a level plain,

Plate 3.5. A demand valve connected to a C size oxygen cyclinder. The cylinder, valves and other emergency equipment were carried in the small back-pack pictured. An intubated cow, wedged in a wallow, is pictured in the background. Two regulators were required; one (pictured attached to the cyclinder) which attached to a C size cylinder, and another (lower left) which attached to D, E and G size cylinders.

with the neck extended, appeared to give the largest excursions of the body wall, and were presumably the most efficient.

Assisted ventilation was performed on all animals which could be intubated and appeared to have difficulty breathing or were at levels of chemical restraint > 5. If animals with breathing difficulties could not be ventilated due to insufficient chemical restraint diazepam, suxamethonium, or further anaesthetic was administered. This appeared to improve breathing, and allowed access to the upper respiratory tract so that assisted or controlled ventilation could be performed. It also appeared to improve the effectiveness of ventilation*.

Morphometrics

Length/ girth measurements

Length was measured as a straight line from the tip of the nose or proboscis to the tip of the tail using a tape measure and was referred to as the snout-tail length. Girth was measured immediately behind the axillae by rolling the animal over a tape laid out on the ground at 90^{o} to it. Where the terrain or depth of anaesthesia did not allow this, the tape was laid across the animal's back, linking points on the ground directly below the axillae, and the distance between the points measured. This distance was then added to the straight-line distance between the two points directly below the axillae to estimate the girth.

Mass

Once anaesthetised, animals were weighed if terrain and depth of anaesthesia allowed. Two or 3 people were required to roll each animal onto a purpose built sling constructed of 2 aluminium tubes (outside diameter 48 mm; 4.4 mm wall), each 3 m long, between which woven nylon strapping (car seat belt webbing and nylon sheeting) was slung. A tripod, constructed of aluminium tubes 4 m in height, was erected above the animal lying on the sling in sternal recumbency. A 1 tonne mini chain block with 3 m lift was used to suspend the animal from a 2.5-ton digital scale (Plate 3.6).

Where possible animals were weighed; otherwise length-mass relationships determined in Chapter 5 were used to estimate mass.

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* It should also be noted that air can also be used and that the gas diving pressure can be provided by the operator blowing down the tube in animals weighing up to 1000 kg. More than one breath may be required to inflate the lungs. Inflation should be gauged by chest expansion (Fedak personal communication 1990).

Plate 3.6. Weighing a yearling. A tripod this size was used to weigh animals up to large cow size.

Tagging

Animals were identified with blue plastic flipper tags with a 4 digit number embossed on each flange and "Inform Antarctic Australia" on the reverse side of the female flange. Tags were inserted approximately 2 to 3 cm into the trailing edge of the interdigital webbing between DI and DII with the male flange on the plantar surface. It was believed that by locating the male flange on the plantar surface the sharp point of the flange (having been punched through the flipper to the dorsal surface) would not excoriate the plantar surface of the opposite flipper when the flippers were opposed. Tagging occurred at level 3 restraint (heavy sedation) or deeper.

Classification of animals

i. Sex and age

Categorisation of animals was based on that of Laws (1956a), Carrick *et al.* (1962) and Hindell and Burton (1988).

Adult males were categorized as (A) young males (too young to take an active part in breeding, 6 to 8 years old), (B) bachelor bulls (too young or too small to compete in harems) and (C) breeding males within or competing for harems, either beachmasters or challengers. Allocation of males to bachelor- and breeding-male categories was simple during the breeding season when their behaviour differed markedly, but at other times these animals were simply defined as adult males. Juveniles were defined as animals that had not reached physiological maturity, < 3 - 4 years for females and < 6 years for males (Carrick et al., 1962). This was judged on the basis of body length for both sexes (Laws 1956a).

ii. Stage of yearly cycle

Four stages of yearly cycle were recognised.

Pre-breeding - pre-breeding cows were within an estimated two to three days of birth. The Ethics committee restricted the study to cows whose pups had died. Each morning, afternoon and evening during the breeding season from September 1 to November 12 each harem on the Isthmus (Carrick *et al.* 1962) was checked for newly dead pups. If a dead pup was found it was dragged out of the harem and deposited in the tussock away from the beach. In this way new pup fatalities could be recognised each day. If the dead pup was still covered with uterine fluid, its nails were still soft and it had a long relatively fresh umbilicus attached, it was considered to have died within 24 hours of birth. Positive identification of the mother of a newly dead pup was thus based on either the death of the pup being witnessed (by crushing or still birth), or the cow having no live pup, actively protecting or nuzzling/ sniffing at the dead pup, or having fresh blood indicating recent birth around the vulva and following the dead pup out of the harem when it was dragged away. If the cow fulfilled these criteria it was marked with yellow yacht enamel paint and its relationship to the dead pup observed over the next 24 hours. If it still remained with the dead pup the cow was used as a subject for pre-breeding

pharmacokinetic studies. Positive identification of mothers was difficult. Often mothers would go to sea or move into the harem before they could be located. Forty seven days were spent checking up to 2700 cows 3 times daily before 5 suitable pre-breeding cows were be located.

Male animals at the start of the breeding season in August, prior to the arrival of the females, were classed as pre-breeding. During September and October they were classed as breeding, and at the end of the breeding season in November, when most of the cows had left, as post-breeding.

Post-breeding - Post-breeding cows were near the end of lactation and within an estimated 3 days of weaning their pups. These animals were usually thin, and often located at the periphery of the harem with large moulted or semi-moulted pups. These animals would have little interest in the pup which would be active and often stray from the mother. The mothers would often be moving toward the sea away from their pup or making repeated glances seaward. Animals witnessed actively moving out of the harem and towards the sea were not used in trials as in most cases they appeared a very poor anaesthetic risk. Immobilization in the intertidal zone for 3h may have risked aspiration of sea water, interference by numerous challenger bulls would have made protection of the animal more difficult and the partially sedated animal on recovery may not have been able to protect itself from challenger and bachelor bulls whilst trying to get to sea. Thus work on the landward side of the periphery of small harems controlled by a stable beachmaster was preferred.

Pre-moult - Pre-moult animals were those at the beginning of the moult and within 3 days after coming ashore. These animals were characterised by a light brown to dark brown/ black coat, good body condition and lack of skin sloughing (Stage 0 - 1; Ling 1965). Often the first signs of moulting, such as loss of hair around the orbits or muzzle, could be seen or the animals were observed coming ashore. Animals which had been ashore for two to three days were preferred as animals that had been seen to haul out or were still wet appeared highly strung, anxious or frightened and in many cases could not be approached closely enough to permit needle placement or panicked and headed for the sea when approached or injected. Animals that had been ashore for several days appeared more relaxed, had moved into the tussock areas and were generally more quiet and tractable.

Post-moult - Post-moult animals were thin animals at the end of the moult that had shed their last year's coat and had almost completely regrown their new coat. They were silver grey with a hair coat approximately 0.5 to 1 cm long and could often be found along the beach tussock margin or on the beach itself. Animals in small groups or, in the case of females, in groups with a large male present, appeared more tractable and were the preferred subjects for trails.

Monitoring

A set of standard monitoring techniques was developed and used in all animals (Chapter 4).

Time zero was defined as the time when injection of the first dose of drugs used for chemical restraint had been completed. All times referred to in the text are presented with reference to this time eg "antagonists were administered at 20 min" means that antagonists were administered 20 min after completion of initial administration of drugs used for chemical restraint.

Once each animal was immobilized a standard monitoring sheet was completed (Figure 4.1; abbreviations and definition of terms used in this sheet are listed in Table 4.1). The following responses and reflexes associated with assessment of level of chemical restraint were recorded at approximately five min intervals from time zero until level of chemical restraint was inadequate to allow safe assessment by the operator:

head response, palpebral response, withdrawal response, caudal flipper response, muscle tone, righting reflex, and capillary refill (Table 4.1).

Once these variables had been recorded an overall assesssment of the level of chemical restraint was made. This was based on the ability to handle the animal, gain intravenous access, take blood, roll or weigh animals and assessment of the above responses and reflexes (Table 4.1).

The times from time zero to deepest level of chemical restraint reached and to recovery (defined as light sedation, level 1) were recorded. Duration of deepest level of chemical restraint reached and level of chemical restraint, and time when the animal first moved forward (level and time of first movement forward) were also recorded.

Periods of apnoea, shaking, vomiting or nystagmus were recorded as were any eventful periods during the anaesthetic episode. Times of sampling or when procedures were performed were also recorded.

Statistical analysis

The Statistica/ Mac™ statistical package (StatSoft, Inc., USA, 1991) was used for most statistical procedures. SAS/ STAT™ for personal computers, version 6 (SAS Institute Inc., USA, 1989), was used to perform two-tailed Fisher's exact tests. Differences were considered significant when $P < 0.05$.

Differences in proportions were compared using χ^2 test. Because most data did not appear to be normally distributed, medians were used to compare groups and tested by Mann-Whitney U test (for

two groups) or Kruskall-Wallis H test (for more than two groups). Relationships were compared using Spearman rank order correlation (r_s) unless predictive equations were required (Chapter 5) and in these cases simple or multiple regression analysis was used after $\log_e$ transformation of data. In Chapter 5, analysis of co-variance (ANCOVA) was used to compared Y intercept and slope between groups. Paired Student's t-tests were used to compare paired data.

Curve fitting (Chapter 7) was performed by least squares non-linear regression using SigmaPlot® transforms and curve fitting software (Jandell Scientific, USA, 1992).

Where applicable, specific statistical tests used in each chapter are presented under "Data analysis" at the end of each "Materials and Methods" section.

Chapter 4: Monitoring during chemical restraint

Introduction

There is little information available on the signs and responses of southern elephant seals during anaesthesia. A standard technique for monitoring anaesthesia in southern elephant seals was required for three reasons.

- To allow comparisons to be made between different anaesthetic agents.
- To improve the safety, controllability and understanding of the anaesthetic episode by allowing detection of undesirable responses to physiological and pharmacological events, facilitating their early prevention or treatment and helping avoid excessively deep anaesthesia (Eicker 1986). It would allow the anaesthesiologist to determine whether the required degree of suppression had been achieved (Lumb and Jones 1984 p493).
- A system of monitoring would also help put anaesthesia into its true perspective as a potentially dangerous and life threatening procedure, reinforcing the need to assess the welfare of the animal diligently throughout the whole period of anaesthesia.

A large number of techniques have been used to monitor anaesthesia in animals (Steffey 1983b, Lumb and Jones 1984 p493, Haskins 1987). A pilot study was performed to develop and select those techniques and signs which would be most useful for gauging anaesthetic depth and response to anaesthesia in southern elephant seals under field conditions, and to use them to develop a standard system of anaesthetic monitoring. As techniques were to be used by people with little experience of seal anaesthetics, simple, practical, uncomplicated techniques were required, using the minimum of equipment, which could be easily applied and understood under field conditions.

Materials and methods

A series of monitoring techniques and signs were assessed in seals at various stages of their life cycle which had been chemically restrained with a combination of either ketamine and diazepam (Chapter 6), or midazolam, pethidine and ketamine (Chapter 9). The monitoring signs and techniques were based on those previously used to gauge anaesthetic depth and response to anaesthesia in southern elephant seals and in other mammals (Steffey 1983 b, Lumb and Jones 1984 p493, Gales and Burton 1987 a, Haskins 1987, Woods *et al.* 1989).

Cardiovascular system

Pulse was searched for by digital palpation along the lateral and medial borders of the digits from both plantar and dorsal surfaces, the interdigital webbing between the digits, along all aspects of the tibia and metatarsus, the ventral midline of the tail, the palmar aspect of the fore flipper, the tongue, the ventral border of the jaw, the maxilla, over the jugular groove and the thoracic inlet.

Heart rate was assessed by watching movement of the curve made by the animal's back, the left and right axillae, the area caudal to the sternum when the animal was in lateral or dorsal recumbency, or the jugular groove. Palpation using the flat of the hand was also used over these areas to feel for heart rate. Auscultation was performed with a stethoscope over both lung fields, and an area just caudal and dorsal to left and right axillae and the thoracic inlet. An oesophageal stethoscope, alone, wrapped in tape or with a stilette, was passed to the level of the heart base. The length of stethoscope to be passed to locate the tip approximately over the heart base was estimated before passage by holding the tip of the stethoscope level with an area just caudal and dorsal to the left axilla and lining the other end up with the tip of the nose where a mark was made on it. The stethoscope was then passed down the oesophagus until this mark lined up with the tip of the nose. Dysrhythmias were assessed visually and by auscultation.

Blood pressure was measured percutaneously or after arterial cut-down using an arterial pressure monitor (U.S. Catherter and Instrument Corporation, Glens Falls, New York, USA)(see Figure 19 -17, Lumb and Jones 1984 p511). This was attached to a three way stop cock, connector tubing and an 18 G, 37 mm needle or 20 G, 67 mm thin-wall catheter outside the needle system (Angiocath, Deseret Company, Sandy, Utah, USA) or, for the area of the thoracic inlet, an 18 G, 90 mm needle filled with heparin solution (20 IU/ mL).

To facilitate location of peripheral arteries for cut-down, fore and hind limbs were collected from naturally deceased animals and potentially useful vessels dissected out after placement of catheters within their lumen or infusion with indian ink.

Heavy immobilization with midazolam, pethidine and ketamine was used in the live animal to allow cut down over areas where these arteries had been located (Chapter 9). These areas included along the medial borders of phalanx 3 on digit one (DI) and DV, on the plantar surface of the hind flipper; just cranial to the termination of the interdigital webbing above the first metatarso-phalangeal joint, and over the medial aspect of the tarso-metatarsus; and on the medial aspect of the fore flipper, between the radius and ulna. Cut-down was also performed over the angle of the jaw, and jugular groove at the left distal neck base just cranial to the thoracic inlet.

Capillary refill was assessed by blanching the mucous membranes at the base of the upper canine and counting the time taken, in seconds, for colour to return to the area.

An attempt was made to measure central venous pressure by jugular cut-down after heavy immobilization (≥ level 5 restraint) with midazolam, pethidine and ketamine using a technique based on that outlined by Haskins (1987) and Sweeney (1974). The animal was placed in right lateral recumbency, the jugular groove located by observation of pulsing associated with heart rate, and an incision made over the caudal end, just above the thoracic inlet. Blubber was incised and blunt dissection used in an attempt to locate the jugular vein.

Mucus membrane colour was visibly assessed.

Respiratory system

Respiratory rate was recorded by watching the chest rise and fall, the external nares open and close, listening for sounds of breathing, by feeling for breath with the palm of the hand or cheek over the nares, or by resting the index finger of the left hand just within the opening of the left nostril.

Respiratory depth and nature of ventilatory effort was subjectively assessed by visual observation based on the degree of chest expansion during inhalation, force of exhalation, time taken for inspiration and expiration, and evidence of stertorous or wheezing breathing sounds. Auscultation of both lung fields and upper airway was performed using a stethoscope.

An attempt was also made to measure tidal and respiratory minute volume after intubation using a Wright respirometer (physiological model, Harris Calorific Company, Cleveland, Ohio, USA) attached to the end of the endotracheal tube or by inserting the inlet into one nostril and holding the other closed.

Protective reflexes such as cough reflex and laryngeal reflex were monitored whilst assessing the ability to intubate immobilized animals.

Attempts were made to collect arterial blood gas samples simultaneously with arterial puncture for blood pressure and pulse measurement. Venous blood gas samples were collected from the extradural intravertebral vein, taking samples every 5 min during the anaesthetic episode until it became dangerous to do so.

Gastrointestinal system

Excessive salivation, vomiting, regurgitation or defecation was noted and the oropharyngeal reflex checked whilst intubating.

Ocular system

Observation of the pupils, corneal reflex in response to touching the cornea with the tip of a pair of curved mosquito haemostats, lacrimation, photomotor reflex using a pen light, palpebral response in response to touching the medial canthus, eyeball position and nystagmus were noted. Closure of eyes in response to touch of the vibrissae located dorsal and medial to the eye was also tested.

Musculoskeletal system

Limb muscle tone (caudal flipper response and withdrawal response), jaw tone, abdominal muscle tone and degree of muscle tone in response to rolling of the animal (righting response), and ability to move the head were used to assess muscle tone. (For definitions of caudal flipper response, withdrawal response, righting response, head response and muscle tone see Table 4.1). Anal tone was assessed during placement of a rectal thermometer.

Nervous system

Sensorium, pedal reflexes including withdrawal response and caudal flipper response, reaction to surgical manipulation, head response and response of muzzle vibrissae to touch were assessed as gauges of the status of the central nervous system.

Thermoregulatory system

Rectal temperature was assessed using a 12 inch glass/mercury rectal thermometer inserted approximately 15 cm into the rectum or a digital thermometer inserted approximately 4 cm into the rectum and by checking for signs of heat stress such as moving into wallows, "sand flicking" or elevation of the temperature of the skin as assessed by laying the palm of the hand on the back.

Results and discussion

General

The most practicable criteria for monitoring anaesthesia in the field were found to be:

Heart rate, respiratory rate, rectal temperature, assessment of reflexes and responses (head response, palpebral response, withdrawal response, caudal flipper response, muscle tone and righting response as well as capillary refill, and degree of shaking) and an overall assessment based on the ability to handle the animal, gain intravenous access, take blood, roll or weigh the animal (Table 4.1).

It was found that level of chemical restraint fell into 9 easily recognisable categories (Table 4.2).

Table 4.1. Definitions used to monitor response to chemical restraint of female southern elephant seals

Response used in monitoring	Definition
Heart rate:	Measured as excursions of the body wall (beats/ min)
Respiratory rate:	Measured as each inspiratory/expiratory cycle per min (breaths/ min)
Rectal temperature:	Assessed using a 12 inch rectal thermometer inserted approximately 15 cm into the rectum
Head response:	A graded response, based on the speed and degree of withdrawal in response to touching the top of the head and the side of the muzzle, and gently lifting up both sides of the muzzle simultaneously
	0 Normal response - cannot approach head without risk of being bitten
	1 Near normal response ie slight slowing of response, high risk of being bitten
	2 Can raise head and shoulders above the ground, rear up, usually bite at operator
	3 Can raise head, often with effort, often allowing it to flop to the ground once stimulus is withdrawn; may bite
	4 Cannot raise head; can move it laterally in horizontal plain away from point of touch on opposite side of muzzle
	5 Cannot raise or pull head away laterally but eyes will follow path of operator walking past the head
	6 Eyes won't follow path of operator walking past the head
Palpebral response:	A graded response based on the speed of closure of the lids in response to touching the medial canthus
	0 Lost
	1 Sluggish
	2 Moderate
	3 Brisk (near normal)
Withdrawal response:	A graded response, based on the speed and degree of withdrawal of the fore-limb and body in response to pinching the nail bed of DII of the left fore-flipper
	0 Lost
	1 Sluggish
	2 Moderate
	3 Brisk (near normal)

Table 4.1. (Continued). Definitions used to monitor response to chemical restraint of female southern elephant seals

Response used in monitoring	Definition
Caudal flipper response:	A graded response, based on the speed and degree of movement of the caudal flukes, hind quarters and body in response to hand pressure applied at the level of phalanx 1 and 2 across the width of DI of the left caudal fluke.
	0 Lost
	1 Sluggish
	2 Moderate
	3 Brisk (near normal)
Muscle tone:	Degree of muscle tone in response to rolling, or attempting to roll the animal
	0 Lost (flaccid)
	1 Slight
	2 Moderate
	3 Near normal
Righting response:	Ability to maintain sternal recumbency when attempting to roll the animal over
	0 Lost
	1 Slight, muscle tone increased in response to rolling but able to roll
	2 Moderate/ fighting (but able to roll over)
	3 Near normal, can't roll
Capillary refill:	Time in seconds for colour to return to blanched area of mucus membrane above upper canine
Shaking:	Degree of shaking
	1 Slight/ fine
	2 Coarse
	3 Spasms (clonic-tonic)
	4 Status epilepticus

Table 4.2. Definitions of levels of chemical restraint

Level of chemical restraint*			Definition
0	Normal behaviour		Normal behavioural responses
1	Sedation	Light	General slowing of behavioural responses, able to move away
2		Moderate	Able to rear up, will move away or swivel round in response to touch
			Brisk palpebral response
			Brisk caudal flipper response and withdrawal response
			Free movement of head, able to bite operator
3		Heavy	Marked slowing of behavioural responses
			Able to raise head when simultaneously lift both sides of muzzle
			gently upwards but generally won't bite operator
			Sluggish response to tactile stimuli
			Righting response still present and can only rarely be rolled over onto a sling for
			weighing. Caudal flipper response and withdrawal response slowing
			Unable to swivel round and unlikely to move away in response to touch
4	Immobilization	Light	Conscious but only able to move head laterally several cm from point of touch
			Withdrawal response present
			Muscle tone pronounced and will often form a U shape when rolling onto a sling is
			attempted, and may paddle with fore-flippers
			Rolling is difficult but can be carried out
			Brisk palpebral response

*Overall assessment of the level of chemical restraint based on the ability to handle the animal, gain intravenous access, take blood, roll or weigh the animal and assessment of reflexes and responses.

Table 4.2. (Continued). Definitions of levels of chemical restraint

Level of chemical restraint*			Definition
5		Heavy	Unable to move head away from point of touch however eyes may follow objects passing within the field of vision Moderate palpebral response Righting response small, muscle tone slight but animal can be rolled over
6	Anaesthesia	Light	Unconscious, and little response to tactile stimuli loss righting response sluggish palpebral response Loss withdrawal response Loss muscle tone
7		Moderate	Very hard to determine this depth however respiratory rate may drop or become more laboured/ apneustic
8		Deep	Little or no palpebral response Complete loss of muscle tone, totally flaccid, "blob of jelly" like with muscle slumping to appear pear shaped from head on. Heart and respiratory rate may be slow and irregular; often there is apnoea

*Overall assessment of the level of chemical restraint based on the ability to handle the animal, gain intravenous access, take blood, roll or weigh the animal and assessment of reflexes and responses.

A standard monitoring sheet was made up to record the anaesthetic episode based upon regular observation of these variables (Figure 4.1). Columns were also inserted in the monitoring sheet to allow comments to be made at various times, such as presence of nystagmus or vomiting, or the use of mechanical or assisted ventilation, intubation, any further drugs administered and times of blood sampling. This system of monitoring was used throughout this thesis.

Cardiovascular system

In mature animals a peripheral pulse could not reliably be located by percutaneous digital palpation at any of the locations tried. In pups and weaners a pulse could occasionally be located on the medial aspect of the forelimb, in the groove between the ulna and radius. The inability to repeatedly palpate a pulse was exacerbated by the cold which made the operator's finger-tips less sensitive.

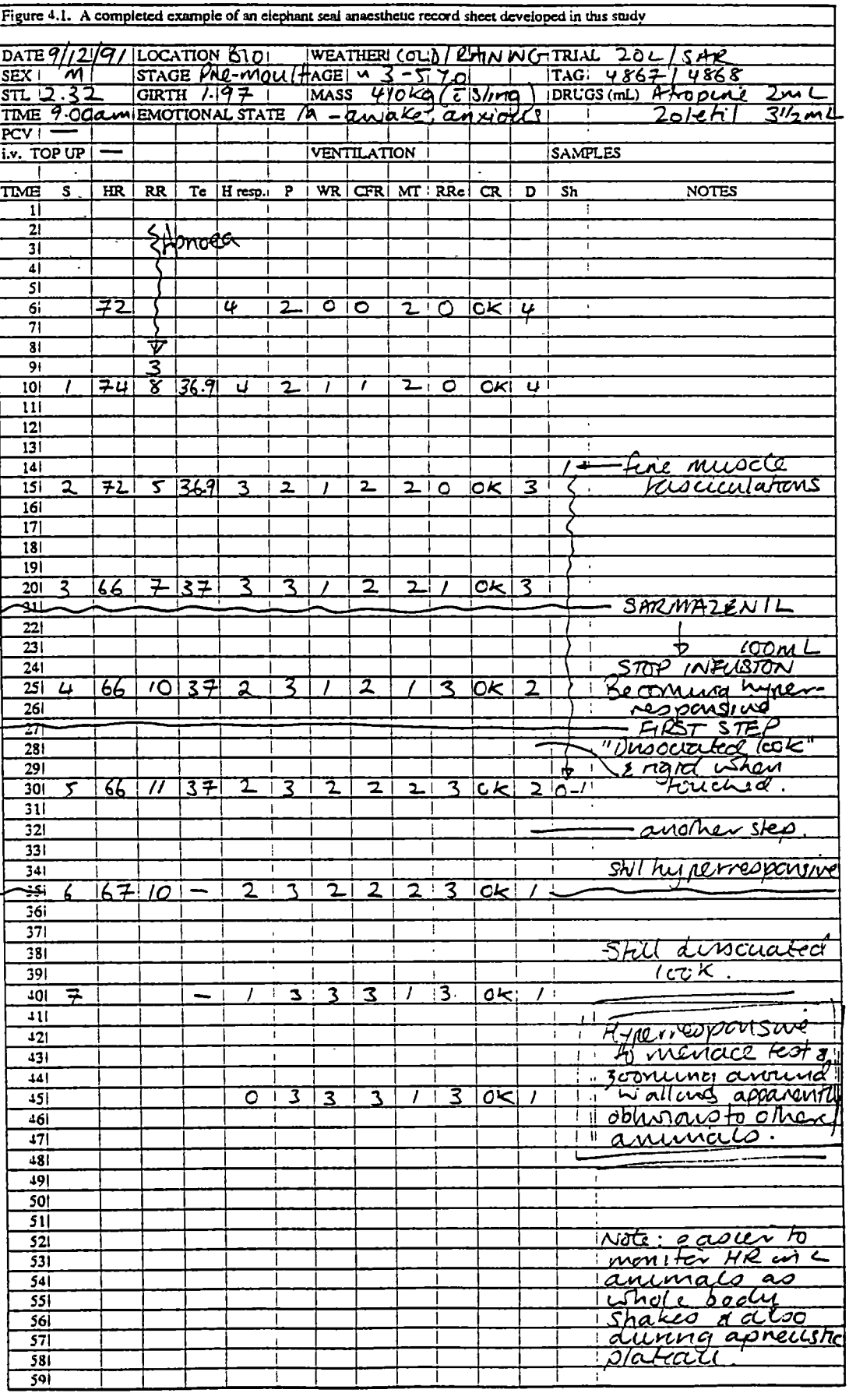
Dissection of peripheral arteries of the fore and hind limbs revealed three potentially useful sites for pulse detection and arterial puncture after cut-down: along the medial border of phalanx 1 of DV and along the medial border of phalanx 1 of DV and metatarsal 5 (MTV), on the plantar surface of the hind flipper (common plantar digital arteries); just cranial to the termination of the interdigital webbing above the first metatarso-phalangeal joint, and over the medial aspect of the tarso-metatarsus (medial plantar artery)(see Figure 18. St. Pierre 1974); and on the palmar aspect of the fore flipper, between the radius and ulna (median artery)(see Figure 22. St. Pierre 1974).

These arteries have not previously been described in southern elephant seals but their location appeared similar to those described in the harp seal (*Pagophilus groenlandicus*) (St. Pierre 1974) and for convenience similar nomenculature was used to describe them. They were difficult to locate and smaller than expected in the flippers collected from dead animals. In the live animal they were found to be relatively deep, small, hard to visualise and a pulse was detected intermittently with difficulty. Difficulty in detecting a pulse in these vessels, even with cut-down, may have been associated with partial or complete peripheral shut down associated with heat conservation or apnoea.

The facial artery and pulse could not be located after palpation of the ventral border and maxilla of the jaw. A strong pulse could be detected after cut down over the jugular groove at the left distal neck base just cranial to the thoracic inlet. However, due to the depth of blubber and bleeding in the area, vessels were not visualised. A lingual pulse could not be located and it was concluded that in mature animals use of peripheral pulse for monitoring was impractical.

Heart rate, in most cases, could be monitored by watching movement of the curve made by the dorsal body wall, the left or right axillae, the area caudal to the sternum when the animal was in lateral or dorsal recumbency, or the jugular groove (palpation using the flat of the hand, or leaning with the back against the chest wall also could detect a heart beat in some cases). However, in some cases

Figure 4.1. A completed example of an elephant seal anaesthetic record sheet developed in this study



DATE 9/12/91 | LOCATION BIO | WEATHER (COLD) RAINING | TRIAL 20L / SAR
SEX M | STAGE Pre-moult | AGE ~ 3-5 Y.O | TAG: 4867 / 4868
STL 2.32 | GIRTH 1.97 | MASS 410kg (± 5kg) | DRUGS (mL) Atropine 2mL
TIME 9.00am | EMOTIONAL STATE A - awake, anxious | Zoletil 3½mL
PCV —
i.v. TOP UP — | VENTILATION | SAMPLES

TIME	S	HR	RR	Te	H resp.	P	WR	CFR	MT	RRe	CR	D	Sh	NOTES
1			Apnoea											
2														
3														
4														
5														
6		72			4	2	0	0	2	0	OK	4		
7														
8			▽											
9			3											
10	1	74	8	36.9	4	2	1	1	2	0	OK	4		
11														
12														
13														
14													1	fine muscle fasciculations
15	2	72	5	36.9	3	2	1	2	2	0	OK	3		
16														
17														
18														
19														
20	3	66	7	37	3	3	1	2	2	1	OK	3		
21														SARMAZENIL
22														
23														100mL
24														STOP INFUSION
25	4	66	10	37	2	3	1	2	1	3	OK	2		Becoming hyper-responsive
26														
27														FIRST STEP
28														"Dissociated look"
29														& rigid when touched.
30	5	66	11	37	2	3	2	2	2	3	OK	2	0-1	
31														
32														another step.
33														
34														Still hyperresponsive
35	6	67	10	—	2	3	2	2	2	3	OK	1		
36														
37														
38														Still dissociated look.
39														
40	7			—	1	3	3	3	1	3	OK	1		
41														
42														Hyperresponsive to menace test & becoming aware walking apparently oblivious to other animals.
43														
44														
45					0	3	3	3	1	3	OK	1		
46														
47														
48														
49														
50														
51														
52														Note: easier to monitor HR in animals as whole body shakes & also during apneustic plateau.
53														
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TIME	S	HR	RR	Te	H resp	PR	WR	CFR	MT	RRe	CR	D	Sh	NOTES
60														
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Stage	=	stage of yearly cycle		WR	=	Withdrawal response	
STL	=	snout-tail length (m)		CFR	=	Caudal flipper response	
PCV	=	Packed cell volume		MT	=	Muscle tone	
S	=	Sample		RRe	=	Righting response	
HR	=	Heart rate		CR	=	Capillary refill/ mucus membrane colour	
RR	=	Respiratory rate		D	=	Level chemical restraint	
Te	=	Rectal temperature		Sh	=	Shaking	
P	=	Palpebral response					

heart beat could not be detected: specifically, if animals were shaking associated with anaesthesia or if the silhouette of the animal was not clear, for example when the animal was located within a group of other animals. Hear rate was also hard to detect when animals were lying against or on top of each other as movement of the body wall tended to be difficult to determine against the background, or movement of, other animals.

A heart beat could not be auscultated using a stethoscope. This was exacerbated by wind which, even on relatively still days caused "roaring" in the stethoscope.

Heart rate could be detected in many cases using an oesophageal stethoscope. However in many cases an oesophageal stethoscope could not be passed as animals were too lightly anaesthetised, passage caused apnoea, or the tube could not be passed as it was not rigid enough. Tape wrapped around the tube did not give sufficient rigidity and a stylet generally could not be withdrawn without withdrawing the length of the tube.

It was concluded that heart rate could be measured routinely more effectively by visualising movement of the body wall than by auscultation or use of an oesophageal stethoscope.

Blood pressure was difficult to measure. Percutaneous arterial puncture was successful on one occasion (giving repeated estimations of systolic pressures of 90 to 105 mmHg and diastolic pressures of 65 to 85 mmHg), however it was made difficult by lack of palpable pulse and risk of arteriovenous anastamosis catheterisation. The depth, size, location of vessels and arteriovenous anastomoses prevented routine catheterisation after arterial cut down. Other important limiting factors to this technique for routine monitoring in the field included animals being too lightly immobilized to allow tail manipulation (even slight movement of the tail would made catheter placement difficult), peripheral shut-down of vessels making their localization difficult, and locating the vessels and closure of the skin were time consuming (an important consideration when drugs with a rapid termination of effect were used). An incision on the palmar aspect of the fore flipper would be in contact with the ground when the animal moved away, increasing the chance of infection. Manipulation of catheters, instruments and flushing of catheters was difficult under cold conditions. For these reasons it was concluded that routine monitoring of blood pressure was impractical under field conditions using injectable anaesthetics for procedures of short duration. However, arterial access and blood pressure and blood gas monitoring could be very useful under more controlled conditions or if gaseous, or more prolonged anaesthesia was used, by catheterising the abdominal aorta via a hind flipper artery (see Figure 10., Elsner 1969). Introduction of a 25 G needle into the lingual artery was not attempted due to difficulty in locating a pulse and light levels of chemical restraint making work in that area dangerous in some circumstances. This technique however could be useful if animals were at greater levels of chemical restraint and the lingual arterial pulse could be located.

Capillary refill was found to be a useful estimate of peripheral perfusion but central venous pressure monitoring was unsuccessful due to insufficient time to place catheters and lack of land marks. Passage of catheters via the readily accessible plantar venous arch was not attempted but could be of use for central venous pressure monitoring if pressure changes in this vessel could be correlated with pressure changes within the anterior vena cava.

Respiratory system

Respiratory rate could usually be determined by watching the chest rise and fall, the external nares open and close, listening for sounds of breathing or by feeling for breath by placing the palm of the hand or cheek over the nares, or by resting the index finger of the left hand just within the opening of the left nostril. It was, in some cases, difficult to determine if air was being effectively inspired or shunted in the dead space due to very shallow breathing (this was especially evident when using pethidine based drug combinations). During apnoea the external nares would be held tightly shut. Often the first evidence that an animal was about to commence breathing was twitching of the nostrils and facial musculature. Respiratory depth and nature of ventilatory effort could visually assessed based on the degree of chest expansion during inhalation, force of exhalation, time taken for inspiration and expiration, and evidence of stertorous (indicating upper airway obstruction) or wheezing breathing sounds (thought to be associated with bronchial spasm). Upper airway obstruction (such as laryngeal spasm) was sometimes associated with neck-arching and/ or heaving respiratory effort, whilst the nares were closed or open, with no evidence of inspiration. Auscultation of both lung fields and upper airway was unrewarding due to wind causing "roaring" in the stethoscope.

Breathing stopped when the Wright respirometer was inserted into one nostril or, when it was attached to an endotracheal tube and the animal intubated. Once the respirometer or endotracheal tube and respirometer were removed breathing commenced. The Wright respirometer has minimal resistance to flow so it is unlikely that this was causing apnoea. It was considered more likely that the presence of these devices as foreign bodies in the upper airway may have been affecting breathing. The respirometer was damaged during removal and not used again. Because an endotracheal tube had to be passed and the respirometer attached, and because of the effects it may have had on breathing, it was concluded that for routine procedures it was not useful for monitoring. However it could be useful under more controlled conditions if intubation and gaseous anaesthesia were used.

Protective reflexes such as cough reflex and laryngeal reflex could only be assessed whilst the operator's hand or arm was in the animal's mouth. Animals would often bite down on the operator's arm whilst testing these reflexes so their use under field conditions for routine monitoring was considered impractical. Apnoea was also seen associated with manipulation of the larynx or when passing oesophageal stethoscopes in lightly immobilized animals (level 4 restraint) which could also limit their usefulness for routine monitoring of light levels of chemical restraint (≤ level 5 restraint). However it was found that the presence or absence of these responses when intubating animals gave

useful information on level of chemical restraint; presence of reflexes indicated light levels of chemical restraint (≤ level 5 restraint) whereas loss of reflexes was of concern and usually indicated excessive level of chemical restraint (> level 5 restraint).

Mucous membrane colour was easily assessed but did not appear to relate well with duration of apnoea (presumably reflecting differences in the appropriateness of apnoeic responses (Mitchell and Burton 1991): pink possibly indicating an appropriate apnoeic response and early cyanosis an inappropriate response, see Chapter 13: Apnoea during chemical restraint). However, mucous membrane colour was easily assessed, and when combined with assessment of heart rate, respiratory rate and duration of apnoea was a useful aid in assessment of the status of peripheral oxygenation and perfusion. Cyanotic mucous membranes were of concern during anaesthesia.

Collection of arterial blood gas samples simultaneously with arterial puncture for blood pressure and pulse measurement was difficult due to light levels of restraint (≤ level 4 restraint) and excluded it from routine monitoring when using injectable agents for short term procedures. In situations where the level of chemical restraint was greater for longer periods it could be useful. Venous blood gas samples were easily collected from the extradural intravertebral vein and could be a useful gauge in assessment of response to anaesthesia. However the equipment required and knowledge of its use are limiting factors for its practicality. A potentially more practical alternative which was not assessed was the use of pulse oximetry on the tongue. Of all the techniques used this has the most potential for use and it is recommended that future studies examine its use.

Gastrointestinal system

Excessive salivation, vomiting, regurgitation or defecation could be noted, however due to the anatomy of the oropharynx it was found that vomit was held in the oropharynx and could not always be seen. Animals that had vomited would not breathe until close to death (level 8 restraint) unless the vomit was removed. For this reason a hand was inserted past the sphincter made by the soft palate and the tongue to feel for the presence of vomit rather than relying upon visual assessment or reflux of vomited material through the nose. Vomiting was sometimes difficult to differentiate from laryngeal spasm as the apnoea and heaving of the body appeared similar. For this reason it was considered important to physically palpate the oropharynx and arytenoids to differentiate the two conditions. Regurgitation was also seen, often associated with insertion of the hand into the oropharynx (for passage of an endotracheal tube or gastric lavage tube). The first sign of regurgitation was usually the feeling of hot fluid on the operators cold hand. This usually occurred at excessive levels of chemical restraint (> level 5 restraint). The oropharyngeal reflex could only be checked with the arm in the animal's mouth whilst intubating which made its routine use impractical.

Ocular system

Pupils remained constricted through all episodes of chemical restraint until the animal was near death so observation of the pupils and photomotor reflex were not considered useful for monitoring except in apnoeic animals. In these animals the pupils dilated when terminal cerebral anoxia was near. At this point pupillary dilation was a very useful gauge of the response of the animal to treatment for anoxia. As the operator was ventilating the animal the pupils could be watched and changes in their diameter noted. Any dilation from the normal slit like pupil was a sign of impending death and a sign that blood and tissue oxygenation had to be immediately improved. Usually the more aggressive and rapid the response with mechanical ventilation the easier it was to treat these animals. Pupil dilation could be stopped and oxygenation returned until pupils were approximately greater than 1 cm across the diameter of the elipse. Once the pupils dilated past this point recovery was unlikely. The rapidity with which the pupils dilated was remarkable, occuring over approximately 10 to 30s so the person monitoring the animal had to watch the eyes constantly. By sitting at the front of the animal the pupils could be observed, the animal ventilated, and the nares observed for impending return to normal breathing. In cases where there was concern for the life of the animal it was found more useful just to monitor these signs with heart rate, capillary refill and mucous membrane colour to assess the animal's condition than to routinely assess the other signs of response to chemical restraint.

Corneal reflex was not considered useful due to the number of times the cornea would have to be touched perhaps increasing the chance of corneal damage. Palpebral reflex was more useful. Because eyeballs usually remained fixed in position and nystagmus was noted only occasionally, eyeball location was not considered a useful gauge of anaesthetic depth. Closure of eyes in response to touch of the vibrissae located dorsal and medial to the eye was useful in those animals with vibrissae but not in animals during moult which lose these vibrissae.

Musculoskeletal system

Limb muscle tone (caudal flipper response and withdrawal response), jaw tone, head response, abdominal muscle tone and degree of muscle tone in response to rolling of the animal were useful in assessing muscle tone. Large seals (> 1000 kg) appeared to react more sluggishly to nociceptive stimuli aimed at eliciting these responses which should be considered when assessing the level of restraint in bulls prior to potentially dangerous procedures and greater pressure or more force used. Anal tone was less useful due to intermittent use of a thermometer which could only remain in place up to level 3 to 4 restraint before movement would make its withdrawal necessary.

Nervous system

Withdrawal response and caudal flipper response, were useful gauges of level of chemical restraint. Reaction to surgical manipulation was less useful due to the lack of surgical intervention and concurrent use of local anaesthesia during minor surgical procedures. Response of muzzle vibrissae to touch was useful but inconsistent.

Thermoregulatory system

Rectal temperature assessment using a 12 inch rectal thermometer was useful but in many cases level of chemical restraint was insufficient to allow temperature measurement, and temperatures could not be measured after recovery. Use of a rectal probe attached to a telethermometer such as that used by Mitchell and Burton (1991)(model YSI 401 telethermometer; Yellow Springs Instruments), would improve the ease of monitoring rectal temperature. Telethermometers were not used in this study due to a lack of funding. Use of oesophgageal thermometers was not assessed. These thermometers may allow a more accurate assessment of core temperature, however given the possibility of foreign bodies in the upper airway affecting breathing and it may be wise to approach their use at low levels of restraint with caution.

Temperatures recorded simulataneously by the digital thermometer inserted approximately 4 cm into the rectum gave lower readings than for the 12 inch thermometer and for this reason use of these thermometers was not considered appropriate.

Monitoring during apnoea

It was found that monitoring anaesthesia in the apnoeic patient was more difficult than in the breathing patient. Animals often appeared to be consciously breath-holding. In many cases they appeared to be sedated but refused to move away in response to caudal flipper pressure. However, once breathing commenced the animal would suddenly move off. There thus appeared to be some effect of apnoea on response of the animals to anaesthesia, in most cases making the animals appear more deeply anaesthetised than they really were. This was believed to be a conscious "shutting down" or "dissociation" from their environment which might have been associated with fright in response to light levels of restraint as it was usually only seen in animals at low levels of restraint ($\leq$ level 5 restraint). Testing of this hypothesis would be interesting and further information could possibly be gained by examination of changes in the cortical EEG of these animals during these periods and at other times during the anaesthetic episode.

Bulls, pethidine and unpredictable responses

Responses were harder to elicit in bulls and animals restrained with drug combinations containing pethidine. This was an important finding as these animals would appear to be at greater levels of restraint than they in fact were, which made approaching them dangerous in some cases (for example when intubating). For this reason these animals were approached more cautiously and greater efforts

were used to assess reflexes and responses. In other studies using xylazine or medetomidine, animals also appeared deeper than they really were and could react aggressively and unpredictably to monitoring of the head response (Chapter 8 and 10). These animals also needed to be approached with caution.

Monitoring of cyclohexamine anaesthesia differs from monitoring using non-cyclohexamine drugs and initially may appear difficult as most people will have had experience based upon the responses and reflex changes seen during stages of anaesthesia described for non-cyclohexamine drugs, stages which may not be seen when using ketamine (for a discussion of the conventional stages of general or surgical anaesthesia see Booth 1982 e). The system of monitoring used in this thesis emphasised the lower levels of restraint because of the dissociated nature of the animals response to cyclohexamine drugs and low drug dosages administered. However, the techniques used for monitoring were simple, practical, uncomplicated, used the minimum of equipment and were easily applied and understood. This allowed the level of chemical restraint of the animal and status of the respiratory, cardiovascular and thermoregulatory systems to be rapidly and accurately assessed. Five people with no prior experience with chemical restraint used this system to monitor level of chemical restraint within days of being introduced to it. Though initially appearing complex it was soon understood and in all cases improved understanding of the value of monitoring, the response of animals to various anaesthetic agents, and supplied a common vocabulary with which to discuss and compare the responses of animals to anaesthetic agents.

Chapter 5: Length-mass relationships

Introduction

The mass of an animal is a fundamental parameter in many types of biological investigation (for example growth, energetics and morphometric studies) and an accurate estimate of mass is often also required to ensure safe delivery of anaesthetic drugs. However, for large animals such as southern elephant seals, logistical problems can prevent weighing under field conditions and for this reason relationships based on length and/ or girth have often been used to estimate mass (Ling *et al.* 1967, Ling and Bryden 1981, Gales and Burton 1987a). In previous studies, the relationships were based on small sample sizes, only Gales and Burton (1987a) included quantitative measures of the amount of variance explained by the models, and none provided estimates of variation about the variables in the equations.

Southern elephant seals undergo large changes in mass through various stages of their life cycle. Most of their life cycle is spent at sea, however they come ashore twice each year, once to breed and once to moult. During these periods the animals do not feed and can lose up to 50% of their body mass, mostly as fat (Gales and Burton 1987b). Thus stage in the yearly cycle determines some morphometric parameters of southern elephant seals.

These factors have lead to errors in the estimation of mass (Morgan *et al.* 1978, Ryding 1982, Woods *et al.* 1989). The aim of this part of the study is therefore to generate relationships to estimate mass based on larger sample sizes, with stage of life cycle taken into account, which could be used by workers in the field to estimate mass of southern elephant seals.

Methods

Data sets from southern elephant seals weighed at South Georgia (54°19'S 36°25'W; between 1986 and 1991) and Macquarie Island (54°29'S 157°00'E; between 1963 and 1991) were collected*. These data were pooled and subsequently grouped by sex, stage of the yearly cycle and location. Grouping of animals was based on classifications of Laws (1956 b), Carrick *et al.* (1962) and Hindell and Burton (1988). Animals were classified as either male or female and pre- or post- breeding or moulting. Pre-breeding females were those which had just given birth and pre-breeding males were at the beginning of the breeding season; post-breeding animals were nearing the completion of the

* Data sets were made available by Tom Arnbom, Michael M. Bryden, Nick J. Gales, Mark Hindell, John K. Ling, Peter J. Mitchell, David J. Slip and Rupert Woods.

breeding season; pre-moult animals had just commenced moulting; and post-moult animals had completed moulting. The groups used in this study are presented in Table 5.1. Length was measured as a straight line from the tip of the nose or proboscis to the tip of the tail (the snout-tail length). Girth was measured immediately behind the axillae.

Comparison of mass between female animals at Macquarie and South Georgia were made using two way analysis of variance (ANOVA). There were insufficient values recorded to allow comparisons to be made between males from either island.

For each group (Table 5.1) the relationship between: (1) length and girth, (2) mass and length, (3) mass and girth, and (4) mass and girth and length were determined using simple or multiple regression analysis. Comparisons of Y intercept and slope between groups of seals were made using analysis of co-variance (ANCOVA).

Usefulness of regression equations for predicting mass

The usefulness of the relationships for estimating mass was tested by comparing masses estimated from morphometric data with actual masses for post-breeding and moulting females weighed at Heard Island (53°00'S 73°30'E) in 1987 and 1993, using paired Student's t-tests. The moulting females were weighed at any stage of the moult and therefore could not be assigned to pre- or post-moult stage.

Data analyses

All data were $\log_e$ transformed prior to analysis. Many of the seals were weighed on more than one occassion, usually before and after breeding or before and after moulting, violating the assumption of independence of observations. These animals were randomly allocated to one group or another. Differences were considered significant when $P < 0.05$.

Results

A total of 784 records of mass, length and girth was used. At Macquarie Island 294 records were collected, 430 were from South Georgia, and 60 were collected at Heard Island.

Mass comparions between Macquarie Island and South Georgia seals

The masses of females at Macquarie and South Georgia were not significantly different ($F_{1,493} = 1.781$, $P > 0.05$). Mass varied with stage of yearly cycle ($F_{3,493} = 39.48$, $P < 0.001$) and there was a significant interaction effect between status and location ($F_{3,493} = 3.82$, $P < 0.05$) (Figure 5.1). The interaction effect was due to the pre-breeding Macquarie group (551 ± 112 kg, n = 19) (mean ± standard deviation) being heavier than the South Georgia group (514 ± 116, n = 156). The percentage

Table 5.1. Regression equations in southern elephant seals. Data were Log e transformed prior to analysis. For all relationships P < 0.05. (Y = Y intercept).

Location	Stage of yearly cycle	Relationship	Females						Males					
			Y	Slope		r2	SY.X.	n	Y	Slope		r2	SY.X.	n
				Girth coefficient	Length coefficient					Girth coefficient	Length coefficient			
Macquarie Island and South Georgia	All	Length-girth	0.499		0.645	0.550	0.086	538	0.301		0.878	0.960	0.074	80
		length-mass	3.676		2.546	0.774	0.171	679	3.403		2.950	0.981	0.159	102
		mass-girth	4.574		2.263	0.790	0.172	538	4.231		2.705	0.969	0.201	81
		Multiple	3.831	1.303	1.488	0.906	0.115	538	3.608	0.885	2.072	0.986	0.133	80
Macquarie Island and South Georgia	Pre-breeding	Length-girth	0.547		0.550	0.511	0.054	157						
		length-mass	3.833		2.536	0.773	0.105	189						
		mass-girth	4.895		1.852	0.724	0.114	157						
		Multiple	4.023	0.975	1.595	0.879	0.076	157						
	Post-breeding	Length-girth	0.619		0.536	0.485	0.053	138						
		length-mass	3.431		2.679	0.717	0.121	179	3.834		2.519	0.705	0.115	12
		mass-girth	4.651		2.217	0.812	0.102	138						
		Multiple	3.752	1.438	1.454	0.919	0.068	138						
	Pre-moult	Length-girth	0.240		1.036	0.815	0.068	122	0.271		0.881	0.971	0.062	38
		length-mass	3.724		2.532	0.924	0.114	161	3.357		3.058	0.988	0.120	38
		mass-girth	4.180		2.873	0.884	0.144	122	4.158		2.728	0.985	0.137	38
		Multiple	3.786	1.173	1.641	0.954	0.091	122	3.693	1.214	1.720	0.994	0.088	38
	Post-moult	Length-girth	0.715		0.387	0.336	0.038	40						
		length-mass	3.750		2.297	0.476	0.112	63						
		mass-girth	4.763		1.965	0.735	0.083	40						
		Multiple	3.766	1.425	1.395	0.844	0.064	40						

Table 5.1. (Continued). Regression equations in southern elephant seals. Data were Log e transformed prior to analysis. For all relationships $P < 0.05$. (Y = Y intercept).

Location	Stage of yearly cycle	Relationship	Females						Males					
			Y	Slope		r2	SY.X.	n	Y	Slope		r2	SY.X.	n
				Girth coefficient	Length coefficient					Girth coefficient	Length coefficient			
South Georgia	Pre-breeding	Length-girth	-0.212		0.975	0.572	0.064	124						
		length-mass	3.699		2.669	0.802	0.100	156						
		mass-girth	4.823		1.974	0.751	0.111	124						
		Multiple	3.920	0.974	1.705	0.895	0.072	124						
	Post-breeding	Length-girth	-0.319		0.946	0.554	0.066	107						
		length-mass	3.393		2.713	0.752	0.115	147	3.834	2.519		0.705	0.115	12
		mass-girth	4.633		2.289	0.847	0.096	107						
		Multiple	3.773	1.449	1.434	0.939	0.061	107						
	Pre-moult	Length-girth	-0.106		0.786	0.905	0.048	26						
		length-mass	3.801		2.468	0.956	0.102	63						
		mass-girth	4.197		2.924	0.962	0.091	26						
		Multiple	4.009	1.773	1.000	0.977	0.071	26						
	Post-moult	Length-girth												
		length-mass	4.000		2.042	0.411	0.114	20						
		mass-girth												
		Multiple												

Table 5.1. (Continued). Regression equations in southern elephant seals. Data were Log e transformed prior to analysis. For all relationships $P < 0.05$. (Y = Y intercept).

Location	Stage of yearly cycle	Relationship	Females						Males					
			Y	Slope		r2	SY.X.	n	Y	Slope		r2	SY.X.	n
				Girth coefficient	Length coefficient					Girth coefficient	Length coefficient			
Macquarie Island	Pre-breeding	Length-girth	-0.115		0.927	0.477	0.078	33						
		length-mass	4.280		2.093	0.689	0.112	33						
		mass-girth	5.031		1.594	0.721	0.107	33						
		Multiple	4.394	0.989	1.176	0.834	0.083	33						
	Post-breeding	Length-girth	-0.306		0.985	0.380	0.069	31						
		length-mass	3.429		2.714	0.515	0.143	32						
		mass-girth	-1.691		0.387	0.804	0.039	31						
		Multiple	4.014	1.707	0.957	0.845	0.081	31						
	Pre-moult	Length-girth	-0.015		0.715	0.690	0.060	96	-0.319		1.137	0.939	0.110	28
		length-mass	3.571		2.690	0.892	0.117	98	3.284		3.117	0.990	0.121	29
		mass-girth	4.092		3.001	0.817	0.154	96	3.983		2.873	0.990	0.112	27
		Multiple	3.576	1.244	1.821	0.943	0.086	96	3.640	1.562	1.445	0.997	0.068	27
	Post-moult	Length-girth	-0.241		0.867	0.336	0.057	40						
		length-mass	3.662		2.387	0.496	0.113	43						
		mass-girth	4.763		1.965	0.735	0.083	40						
		Multiple	3.766	1.425	1.395	0.844	0.064	40						

Table 5.1. (Continued). Regression equations in southern elephant seals. Data were Log e transformed prior to analysis. For all relationships $P < 0.05$. (Y = Y intercept).

Examples

1. To estimate the mass of a mature pre-moult female from Macquarie Island of 2.52m snout-tail length prior to drug administration.

Using simple linear regression: Yi = bXi + a (Yi = dependent variable; mass, b = slope, Xi = independent variable; length, a = Y intercept)

lnMass = (2.690*ln2.52) + 3.571
lnMass = (2.690*0.924) + 3.571
lnMass = 6.057
Mass = 427 kg

or Mass = e^((2.690*ln2.52) + 3.571)
Mass = 2.718^((2.690*ln2.52) + 3.571)
Mass = 427 kg

2. Once immobilized the girth is measured and found to be 2.10 m. To estimate mass using length and girth:

Using multiple linear regression:

lnMass = ((1.821*ln2.52) + (1.244*ln2.10) + 3.576)
lnMass = ((1.683) + (0.923) + 3.576
lnMass = 6.182
Mass = 484 kg

or Mass = e^((1.821*ln2.52) + (1.244*ln2.10) + 3.576)
Mass = 2.718^((1.683) + (0.923) + 3.576
Mass = 484 kg

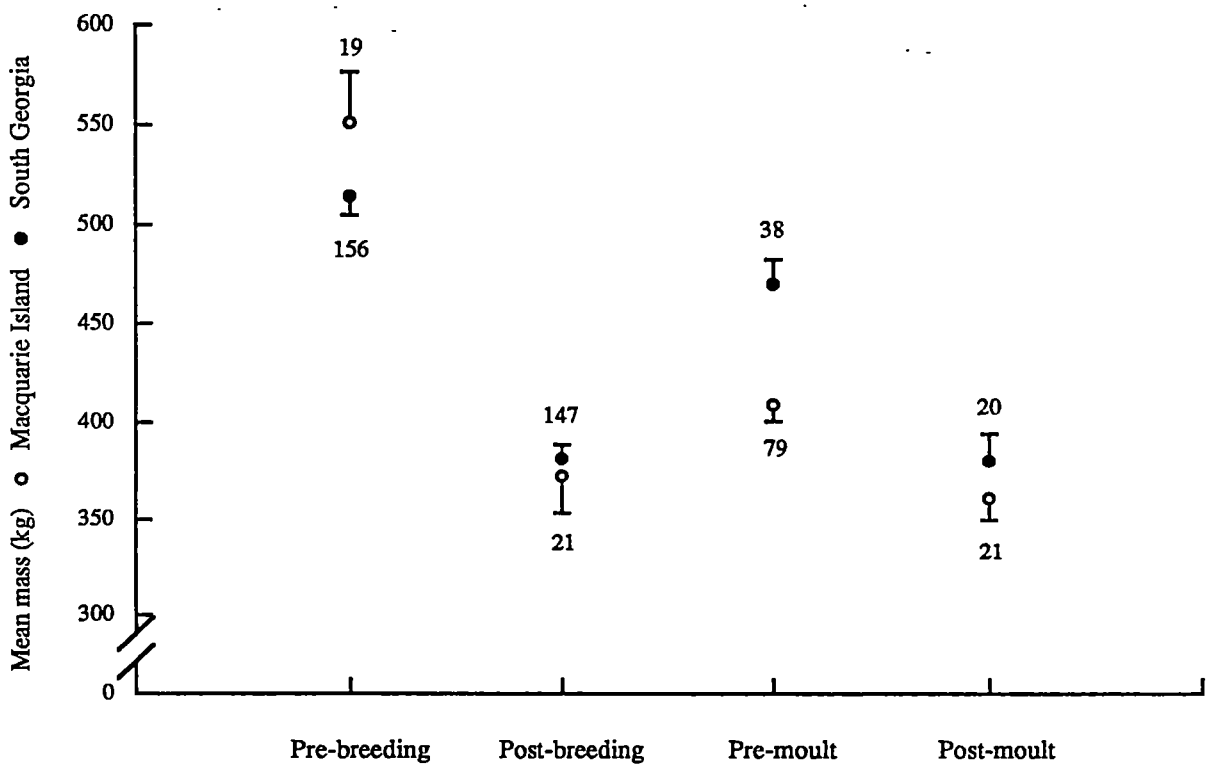

Figure 5.1. The mean mass of mature female southern elephant seals from South Georgia and Macquarie Island at different stages of their yearly cycle. The interaction is due to the pre-breeding Macquarie females being heavier than the South Georgia females. (Error bars are the standard error of the mean. Sample size is also indicated for each location).

mass loss during breeding (mean pre-breeding mass subtract mean post-breeding mass) was 32% for Macquarie Island females and 26% for South Georgia females. Similarly, the percentage mass loss during moulting was 12% for Macquarie females and 19% for South Georgia females.

Descriptive statistics

The derived equations for estimating mass from girth and length, and examples of how they can be used to estimate mass are presented in Table 5.1. Slopes for length-girth relationships in pre- and post-breeding females and Y intercept in post-moult females were significantly different between animals at Macquarie and South Georgia (Table 5.2). Length-mass relationships were not significantly different in post-breeding, pre-moult and post-moult at the islands but were significantly different for pre-breeding females (Table 5.2). Mass-girth relationships were significantly different in pre-breeding and post-breeding females between the islands (Table 5.2).

Usefulness of regression equations for predicting mass

For post-breeding females two regressions (length-mass for post-breeding females from all locations and multiple regression for post-breeding females from Macquarie Island) gave estimates of mass which were not significantly different from those observed. In all other cases for post-breeding animals from Heard Island, regression equations underestimated mass by approximately 10% (Table 5.3).

All of the regression equations used to predict mass of moulting females gave values which were significantly different from those observed (Table 5.3).

Discussion

Mass comparions between Macquarie Island and South Georgia seals

There was no difference between the mass of female seals from South Georgia or Macquarie. This observation is at odds with observations made in the 1960's that the masses of mature females at Macquarie Island were only 60% of those at the Falkland Islands (Bryden 1968), interpreted by Ling and Bryden (1992) to indicate that female southern elephant seals grow larger at South Georgia than at Macquarie. Because there is no difference between pup birth mass and growth rates between the two islands (Hindell, unpublished), it is likely that the significant interaction effect due to the larger mass of pre-breeding females at Macquarie compared with South Georgia (551 ± 112 kg compared with 514 ± 116 kg) was due to different techniques or times of measuring of animals at both islands, or sampling bias (only 19 animals were measured at Macquarie compared with 156 at South Georgia).

The data also indicated differences in mass loss between females at Macquarie and South Georgia during fasting. (The percentage mass loss during breeding was 32% for Macquarie Island females and

Table 5.2. Comparison of relationships for Macquarie Island and South Georgia females using ANCOVA (df = 1,1). When slopes were significantly different (SD), Y intercepts were not tested. No girths were recorded for South Georgia post-moult females. (NSD = not significantly different, F = F values.)

Stage of yearly cycle	Relationship					
	Length-girth		Length-mass		Mass-girth	
	Slope	Y intercept	Slope	Y intercept	Slope	Y intercept
Pre-breeding	SD F = 119.183 P < 0.001		SD F = 5.095 P < 0.05		SD F = 246.101 P < 0.001	
Post-breeding	SD F = 35.997 P < 0.001		NSD F = 0.0001 P > 0.05	NSD F = 2.311 P > 0.05	SD F = 101.225 P < 0.001	
Pre-moult	NSD F = 0.370 P < 0.001	SD F = 2.311 P > 0.05	NSD F = 3.180 P > 0.05	NSD F = 1.087 P > 0.05	NSD F = 0.460 P > 0.05	NSD F = 0.036 P > 0.05
Post-moult			NSD F = 0.253 P > 0.05	NSD F = 0.253 P > 0.05		

Table 5.3. Results of comparison of observed masses and those predicted using length-mass relationships determined in this study, with animals from Heard Island. Moulting females were at an unknown stage of moult.

Heard Island animal stage of yearly cycle	Regression equation used			t	Degrees freedom	P	Mean difference (± SD) between estimated and observed mass	
	Regression	Location (Macquarie or South Georgia)	Stage of yearly cycle				(kg)	(%)
Post-breeding females	Length-mass	Both	Post-breeding	1.944	6	P > 0.05	- 20.0±27.2	- 6.1±8.3
	Multiple	Macquarie Island	Post-breeding	1.850	6	P > 0.05	- 18.7±26.8	- 5.9±8.1
	Multiple	South Georgia	Post-breeding	3.005	6	P < 0.05	- 33.7±29.7	- 10.4±8.9
	Multiple	Both	Post-breeding	2.736	6	P < 0.05	- 30.3±29.2	- 9.4±8.8
	Length-mass	Both	All	3.562	6	P < 0.05	- 30.7±25.8	- 10.7±7.8
	Multiple	Both	All	3.797	6	P < 0.01	- 41.7±29.0	- 12.9±8.8
Moulting females	Length-mass	Both	pre-moult	3.143	36	P < 0.01	17.6±34.1	4.4±8.8
	Multiple	Both	pre-moult	30.171	36	P < 0.001	180.3±36.4	46.4±3.6
	Multiple	South Georgia	pre-moult	17.913	36	P < 0.001	- 127.6±43.3	- 33.1±10.2
	Multiple	Macquarie Island	pre-moult	29.325	36	P < 0.001	142.6±29.6	36.8±4.2
	Length-mass	Both	post-moult	24.100	36	P < 0.001	128.2±32.3	33.0±5.7
	Multiple	Both	post-moult	8.359	36	P < 0.001	34.5±25.1	8.8±6.4
	Multiple	Macquarie Island	post-moult	8.350	36	P < 0.001	34.6±25.1	8.9±6.4
	Length-mass	Both	All	5.620	36	P < 0.001	30.8±33.4	7.8±8.5
	Multiple	Both	All	2.257	36	P < 0.05	9.6±25.9	2.4±6.9

26% for South Georgia females. Similarly, the percentage mass loss during moulting was 12% for Macquarie females and 19% for South Georgia females.) However, such a difference was not reported in a recent comparison of moulting seals (Hindell *et al.* In press) again suggesting that sampling bias could also be involved.

The lack of significant differences in relationships of length and mass for post-breeding, pre-moult and post-moult females between Macquarie and South Georgia mean that the combined relationships for these stages of the yearly cycle can be used to estimate mass. However, for pre-breeding females, and in animals at other stages of their yearly cycle where there were other differences between slope or Y intercept for other relationships, the estimate can be improved by using the relationship specific to that stage of the yearly cycle and location.

Descriptive statistics

Length and girth were always highly correlated and both terms were included in the model solely to improve predictive ability.

Although girth was often a better predictor of mass than length it is sometimes less practical to use. Girth sometimes cannot be determined prior to chemical restraint of animals and in these circumstances length alone may have to be used to predict mass. Girth photographs and digitization have been used to estimate girth in northern elephant seals (*M. angostirostris*) (Haley *et al.* 1991) but this technique is relatively complex and time consuming.

Overall males yielded better relationships than females. The good fit is partly due to the wide spread of data (from immature animals through to adults). Lack of data prevented the generation of relationships for all classes of male animals. Therefore, until such time as these data become available use of the length-mass relationship presented for post-breeding males could be used for post-moult males and the pre-moult relationship for males for pre-breeding males (Table 5.1).

Though the total data set gives reasonable estimates of mass using length, girth, and length and girth, accounting for 77.4%, 79.0%, 90.6% of the variance respectively for female animals, the standard error about any predicted value is still relatively high. Estimates of mass can therefore be improved by using regression equations for sub-groups of the population, as there were significant differences between these relationships (Table 5.2).

Usefulness of regression equations for predicting mass

Only two regression equations gave reliable estimates when used to predict mass of post-breeding females at Heard Island (Table 5.3). One was the Macquarie Island post-breeding female multiple regression which gave an estimate within 5.9% of the actual mass (which was not significantly different). Interestingly the multiple regression for all post-breeding females gave a poorer estimate of

mass (9.4% under-estimate) as did the multiple regression for females from South Georgia (10.4% under-estimate). Relationships developed for Macquarie animals may therefore be more useful in predicting mass of Heard Island animals than those determined for South Georgia animals.

It was therefore interesting that the combined length-mass relationship for all post-breeding females gave a good estimate of mass. Despite this finding we would caution against the use of length-mass relationships rather than multiple regressions for predicting mass when girth is known.

None of the relationships used to predict the masses of moulting females appeared accurate, under or over-estimating mass by up to 36% of mass. The stage of the yearly life cycle (pre- or post-moult) of the Heard Island animals was not known, this analysis highlights the need to know the stage of the life cycle of the animal before masses can be estimated from equations. For this reason generalized equations which do not take these factors into account, despite high r^2 values and low $S_{y.x}$. values will probably be of only very limited use for predicting mass. This conclusion is supported by the fact that the ANCOVAs indicated that some of the relationships differed between islands.

The amount of error precludes the use of these relationships for estimating mass during energetics studies, and in these studies mass should be determined by weighing. Until more data, or other techniques become available, the length-mass relationships presented here, though crude, are however the best estimates available for estimating mass of southern elephant seals in situations where mass and/ or girth are unknown, for example prior to anaesthetic drug delivery. In these cases the amount of error (for example 10% for pre-moult cows in this study) should be considered. Even in cases where the relationships do appear to give reasonable estimates of mass these relationships have been developed only for animals at a particular stage of their life cycle and not for animals between these stages which needs to be considered.

Chapter 6: Drug dosage and normal responses to ketamine and diazepam

Introduction

General

Anaesthesia of southern elephant seals is complicated by the inability to determine drug dose rates accurately and a lack of knowledge of the normal response of animals to anaesthesia.

At the time of this study ketamine combined with diazepam was most commonly used for chemical restraint of southern elephant seals (Gales and Burton 1987 a, Baker *et al.* 1988, McCann *et al.* 1989). These studies reported drug dose rates and some response data but not normal values during restraint for basic physiological parameters such as heart rate, respiratory rate and rectal temperature. There were also few reports of ketamine and diazepam restraint of particularly large animals (≥ 1000 kg) (Gales and Burton 1987a) or information regarding additional drug administration.

This part of the study presents data on responses to a nominal dose of approximately 3 mg/kg (range = 2 - 4 mg/kg) ketamine combined with 0.03 mg/kg diazepam (range = 0.02 - 0.04 mg/kg) (100:1 ketamine: diazepam) in southern elephant seals. Based on these findings, nominal doses are recommended for use in animals where mass cannot be accurately estimated.

There were two parts to the study.

100:1 ketamine: diazepam dosage

Appropriate drug dosage allows the completion of procedures and minimises problems such as excessive, inadequate or prolonged restraint. A problem associated with drug administration to wild southern elephant seals is the inability to determine mass accurately, and therefore determine the dose, prior to drug administration. Length-mass relationships have been used to estimate mass, but have been found to be inaccurate in some cases leading to problems of inappropriate dosage (Chapter 5; Morgan *et al.* 1978, Ryding 1982, Woods *et al.* 1989). Techniques were needed to improve ketamine and diazepam dosage determination.

Southern elephant seals can readily be categorised according to specific, easily recognisable stages in their life and yearly cycle, their sex, and size (Laws 1956 a, Carrick *et al.* 1962, Hindell and Burton 1988, Woods *et al.* 1989). Similarities in response to anaesthetics within categories lead Woods *et al.* (1989) to suggest nominal doses of ketamine and xylazine.

It was decided to determine nominal doses of a 100:1 ratio of ketamine: diazepam (100:1 ketamine: diazepam) for each category of animal and use this data to develop a guide for drug administration. It was hoped that this would offer a practical alternative to the use of length-mass relationships when calculating 100:1 ketamine: diazepam dosages in animals whose masses were unknown.

The normal response to 100:1 ketamine: diazepam

It is important to understand the normal response to anaesthetics so that the anaesthetic episode can be accurately assessed, abnormalities identified and complications treated.

In other mammals heart rate and respiratory rate decrease as body size increases (Schmidt-Nielsen 1985). The required anaesthetic dose of ketamine is also known to decrease as body size increases in some other animals (Sedgwick 1988). Southern elephant seals vary widely in size. Animals weigh approximately 40 kg at birth and can grow to over 3500 kg (Ling and Bryden 1992). It is not known what effect size has on the response of southern elephant seals to 100:1 ketamine: diazepam. Limited data presented by Ryding (1982) showed that heart rate was lower in 3 large animals (34 - 54 beats/ minute; bpm) than in 2 smaller animals (72 - 74 bpm) which were immobilised with ketamine.

The aim of this part was to determine normal values for time course of chemical restraint and physiological parameters during 100:1 ketamine: diazepam restraint for southern elephant seals.

Materials and methods

General

The study was performed opportunistically on animals being restrained for other purposes.

i. Classification of animals

Southern elephant seals were selected in various stages of their life-cycle. Animals were categorised by sex, age, stage of yearly cycle and morphometric characteristics including size, snout-tail length, and proboscis development (Figure 6.1) (Laws 1956 a, Carrick *et al.* 1962, Hindell and Burton 1988, and Woods *et al.* 1989).

Yearlings were small juvenile animals of approximately 2.0 m snout-tail length that had not reached physiological maturity. Young males were sexually mature but still too young to take an active part in breeding and of approximately the same size range as mature females. (Males of similar size to females were differentiated by external genitalia or tooth morphology; we observed that in males lower canines are conical and peg like, in females they are narrower, less peg like and more hooked). Bachelors were bulls too young or small to compete in harems (snout-tail length ~ 3.5 m, with small proboscis). Breeding males within or competing for harems were divided into challengers (competing

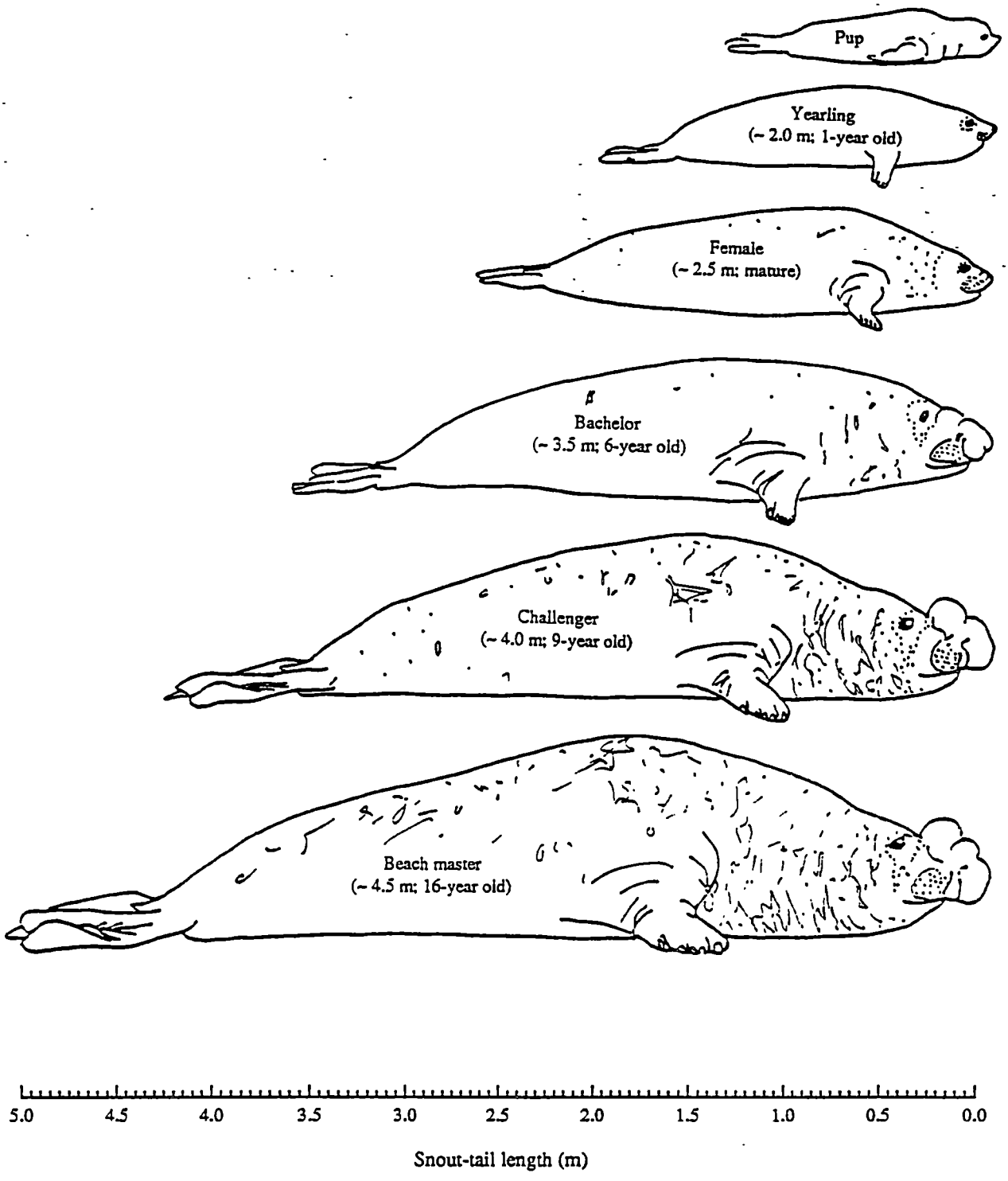

Figure 6.1. Characteristic size, proportions and appearance of southern elephant seals in categories used in this study. Note relative snout-tail length (STL) and proboscis development (From Laws 1956 a). The figures in brackets are approximate STL (m) and age.

for, and located around the periphery of the harem; snout-tail length ~ 4.0 m), and beach masters (located within a harem; snout-tail length ~ 4.5 m) (Figure 6.1). Breeding males had large proboscises.

Animals were divided into pre- and post-fast stages of their yearly cycle. Pre-fasting animals were those which had recently come ashore either to moult or breed. Post-fast animals were those which had been ashore fasting for a considerable period of time and had lost a large proportion of their body mass, mostly as fat, and had completed moulting, were close to weaning a pup, or were at the end of the breeding season. Amongst yearlings, only pre-fasting animals were restrained.

ii. Chemical restraint

Each animal received a single intramuscular dose of 100:1 ketamine: diazepam. Initially the drug dose was based on that found to be useful in pre-moult females (approximately 3 mg/kg (range = 2 - 4 mg/kg) ketamine and 0.03 mg/kg (range = 0.02 - 0.04 mg/kg) diazepam; Chapter 8). The quantity of 100:1 ketamine: diazepam was increased or decreased in subsequent seals until a dose was found which induced level 3 - 4 restraint (Table 4.2) in the majority of animals. (Yearling seals received higher doses aimed at inducing level 4 - 5 restraint so that gastric lavage could be performed). Dose rates were determined in this manner for seals in each category. If animals were insufficiently restrained additional ketamine was administered either intramuscularly or intravenously. Additional doses of ketamine were based on past experience administering ketamine and xylazine.

Animals < 3.50 m snout-tail length received 1.3 mg of atropine sulphate, longer animals received 2.6 mg.

100:1 ketamine: diazepam dosage

Two drug dosages were recorded:

- The actual drug dose (mg/kg) based on mass.
- Additional doses of ketamine (% of initial total dose and mg/kg).

The mass of each animal was determined by weighing, or if this was not possible due to terrain, estimated from the appropriate multiple regression incorporating length and girth (Table 5.1).

At the completion of the trial the nominal dose of drug administered to each category (mg or g) was determined by multiplying the median nominal mass of all animals in each category (to the nearest 50 kg for small animals and 100 kg for large animals) by a nominal initial dose of 3 mg/kg (2 - 4 mg/kg) ketamine and 0.03 mg/kg (0.02 - 0.04 mg/kg) diazepam (found to be effective in most animals; below).

Normal response to 100:1 ketamine: diazepam

A standard monitoring sheet was completed for each animal (Figure 4.1). The level of restraint was determined from this information and graded from 1 to 8 (Table 4.1 and 4.2). At 5 min intervals beginning 10 min after administration of drugs, blood samples were taken to measure blood gases, and each animal's reflexes and responses to stimuli were recorded. The assessment of reflexes and responses continued until the animals had recovered (usually within 90 min). (The technique, and definitions and abbreviations for the variables used for monitoring and blood gas analysis are presented in Chapters 3 and 4). Since several (usually 4 or 5) assessment values were obtained at levels 3 or 4, the median was used to give a single value.

Animals were also assessed for: shaking, apparent hyperthermia (moving into water and/ or flicking sand or water over the body) and other notable occurrences.

Values to describe the normal time course of anaesthesia for each category of animal including maximum level of restraint, time to and duration of this level and time to light sedation were determined from values recorded for each animal from the standard monitoring sheet. Those variables considered most important in monitoring the status of the cardiovascular system (heart rate, capillary refill, mucus membrane colour), respiratory system (respiratory rate) and thermoregulatory system (rectal temperature) were also determined at level 3 - 4 restraint.

Data analysis

Only values for animals which received no other treatment or additional doses of drug were used in the data analysis. Groups were compared using the Mann-Whitney U test, relationships were compared using Spearman rank order correlation (r_S). Differences were considered significant when $P < 0.05$.

Results

100:1 ketamine: diazepam dosage

Two hundred and twenty five animals were immobilized. The actual initial drug doses administered to each category of seal are presented in Table 6.1A. The actual initial drug dose level required to produce level 3 - 4 restraint remained relatively constant as the mass of the animal decreased and the dose of approximately 3 mg/kg ketamine combined with 0.03 mg/kg diazepam induced level 3 - 4 restraint in the majority of animals to which it was administered.

Table 6.1A. Lengths, masses, initial doses of ketamine and diazepam, the maximum level, time and duration of chemical restraint and time to recovery (light sedation, level 1) (median, range) for different classes of southern elephant seal at different times in their life cycle. (Levels of chemical restraint are presented in Table 4.2.)

Category			Snout-tail	Mass	Initial drug dose (mg/kg)		Maximum level of chemical restraint			Time to recovery	n
Sex	Age	Stage of yearly cycle	length (m)	(kg)	Ketamine	Diazepam	Level	Time (minutes)	Duration (minutes)	(minutes)	
Male and female	Yearling		1.88 (1.63 - 2.30)	190 (119 - 417)	5.94 (3.98 - 8.42)	0.08 (0.03 - 0.16)	6 (4 - 6)	7 (1 - 23)	15 (5 - 37)	48 (6 - 94)	32
Female		Prefast	2.55 (2.10 - 2.95)	469 (283 - 700)	2.60 (1.71 - 6.12)	0.05 (0.02 - 0.13)	3 (1 - 8)	10 (1 - 39)	14 (1 - 73)	50 (18 - 125)	73
		Postfast		366 (278 - 576)	2.84 (1.80 - 6.81)	0.03 (0.02 - 0.07)	4 (1 - 6)	7 (1 - 17)	14 (2 - 26)	43 (30 - 66)	46
Male	Bachelor	Prefast	3.82 (3.43 - 4.05)	1855 (1806 - 2038)	2.59 (1.73 - 3.14)	0.02 (0.01 - 0.02)	3 (1 - 3)	9 (9 - 9)	20 (5 - 24)	37 (28 - 53)	5
		Postfast		1334 (1031 - 1567)	2.88 (2.11 - 4.09)	0.04 (0.03 - 0.04)	5 (1 - 5)	16 (15 - 17)	20 (14 - 30)	90 (50 - 110)	15
	Harem bulls										
	I. Challenger	Prefast	4.00 (3.70 - 4.24)	2486 (1574 - 2635)	2.93 (2.43 - 3.66)	0.04 (0.02 - 0.04)	3 (1 - 4)	9 (9 - 16)	20 (9 - 31)	95 (95 - 95)	4
		Postfast		1518 (1334 - 1758)	3.16 (1.48 - 3.81)	0.03 (0.03 - 0.04)	4 (3 - 5)	16 (10 - 43)	15 (6 - 30)	57 (40 - 110)	27
	II. Beach master	Prefast	4.33 (4.00 - 4.81)	2693 (2068 - 2897)	2.32 (1.93 - 2.55)	0.02 (0.01 - 0.05)	3 (2 - 4)	14 (10 - 18)	7 (7 - 8)	63 (21 - 70)	4
		Postfast		1854 (1666 - 2416)	3.02 (1.66 - 3.68)	0.03 (0.02 - 0.05)	3 (2 - 5)	14 (1 - 24)	25 (10 - 48)	61 (34 - 82)	19
All animals					2.87 (1.48 - 8.42)	0.03 (0.01 - 0.16)	4 (1 - 8)	10 (1 - 43)	15 (1 - 73)	50 (6 - 125)	225

Thirty animals required additional doses of ketamine to adjust the level of restraint from 2 to 3 - 4. Five of these animals received a single additional intramuscular dose of ketamine of approximately 50% of the initial dose of ketamine. The other 25 animals received additional ketamine intravenously. Most animals received only 1 additional dose (range = 1 - 8) of approximately 33% of the initial dose of ketamine. Subsequent additional doses were usually smaller. Additional ketamine administration was associated with slowed or laboured breathing or apnoea, and shaking was seen in 1 animal.

Nominal doses of drug for each category of animal based on a nominal dose of 3 mg/kg (2 - 4 mg/kg) ketamine and 0.03 mg/kg (0.02 - 0.04 mg/kg) diazepam and calculated from nominal masses recorded for animals within each category are presented in Table 6.2.

A procedure which could be used to determine 100:1 ketamine: diazepam dose to administer to a southern elephant seal is presented in Figure 6.2.

The normal response to 100:1 ketamine: diazepam

Variables describing the the time course of restraint for each category of seal are presented in Table 6. 1A. As mass increased there was a tendency for the time to maximum level of restraint, its duration, and time to recovery to increase.

Seventy nine animals reached level 3 - 4 without any additional drugs or other treatments. Heart rate and respiratory rate at level 3 or 4 of restraint in these animals tended to decrease as mass increased and heart rate ($U = 133.5$, $P < 0.01$) and respiratory rate ($U = 205.0$, $P < 0.05$) at level 3 or 4 were significantly higher in smaller animals (< 1000 kg) compared with larger animals (≥ 1000 kg) (Table 6.1B).

Normal values, and those considered abnormal for large and small seals are presented in Table 6.3. Values considered abnormal were those seen when animals were either excessively (> level 5) or inadequately (< level 3) restrained.

Pv_{CO2} ($U = 120.0$, $P < 0.01$) and pH ($U = 75.5$, $P < 0.01$) were significantly lower at level 3 or 4 in larger animals compared with smaller animals. Median Pv_{CO2} was 50 mmHg in small animals compared with 57 mmHg in large animals and median pH was 7.35 in small animals and 7.27 in large animals. Pv_{O2} (median = 45 and 39 mmHg respectively) and HCO_3^- (median = 27 and 26 mmHg respectively) were not significantly different between large and small animals at level 3 or 4 restraint. There was a tendency for Pv_{CO2} and HCO_3^- to increase as mass increased ($P < 0.05$), though the relationships were not close ($r_s \leq 0.6$).

Table 6.1B. Heart and respiratory rates (median, range) for different categories of southern elephant seal restrained to level 3 - 4 with a single intramuscular dose of ketamine and diazepam at different times in their life cycle. Values are also presented for small and large seals. (bpm = beats per minute.)

Category			Heart rate at level 3-4 (bpm)	Respiratory rate at level 3-4 (breaths/ minute)	n
Sex	Age	Stage of yearly cycle			
Male and female	Yearling		100 (88 - 104)	9 (6 - 20)	8
Female		Prefast	60 (34 - 100)	5 (1 - 16)	36
		Postfast	56 (36 - 88)	4 (1 - 10)	14
All small (< 1000kg) animals*			60 (34 -104)	5 (1 - 20)	
Male	Bachelor	Prefast	44 (36 - 68)	6 (4 - 10)	3
		Postfast	42 (40 - 44)	4.5 (3 - 6)	3
	Harem bulls				
	I. Challenger	Prefast	60 (60 - 66)	4 (3 - 10)	3
		Postfast	44 (36 - 72)	3 (2 - 4)	3
	II. Beach master	Prefast	54 (48 - 66)	2 (1 - 2)	5
		Postfast	48 (40 - 60)	2 (1 - 5)	4
All large (≥ 1000kg) animals*			48 (36 - 72)	4 (1 - 10)	
Total					79

*Heart and respiratory rates were significantly lower in larger animals compared with smaller animals (see text).

Notes

1. The median rectal temperature at level 3 or 4 for all animals was 37.0°C (36.0-39.1°C). Mucus membrane colour remained pink and capillary refill was < 3s.

Table 6.2. Recommended total initial doses of ketamine and diazepam (median, range) required to induce approximately 10-15 minutes of level 3-4 restraint in each category of seal, based on an approximate dose of 3 mg/kg ketamine (range = 2 -4 mg/kg) and 0.03 mg/kg diazepam (range = 0.02 - 0.04 mg/kg). Nominal values for snout-tail length and mass for each category are also included.

Category			Snout-tail	Mass*	Initial dose of drug	
Sex	Age	Stage of yearly cycle	length (m)	(kg)	ketamine (g)	diazepam (mg)
Male and female	Yearling		2.0	200	0.60 (0.40 - 0.80)	6 (4 - 8)
Female		Prefast	2.5	450	1.35 (0.90 - 1.80)	14 (9 - 18)
		Postfast		350	1.05 (0.70 - 1.40)	11 (7 - 14)
Male	Bachelor	Prefast	3.5	1900	5.70 (3.80 - 7.60)	57 (38 - 76)
		Postfast		1300	3.90 (2.60 - 5.20)	39 (26 - 52)
	Harem bulls					
	I. Challenger	Prefast	4.0	2500	7.50 (5.00 - 10.00)	75 (50 - 100)
		Postfast		1500	4.50 (3.00 - 6.00)	45 (30 - 60)
	II. Beach master	Prefast	4.5	2700	8.10 (5.40 - 10.80)	81 (54 - 108)
		Postfast		1900	5.70 (3.80 - 7.60)	57 (38 - 76)

*Nominal mass. Median values (Table 6.1A) were rounded to the nearest 50kg for small animals and 100kg for large animals.

Notes

1. For subadult males (2.0 - 3.5 m snout-tail length) estimated masses and doses of drug can be based on those used for females of similar size and stage of yearly cycle.
2. Pups at birth weigh approximately 38 kg (Ling and Bryden 1992) and grow to approximately 128 kg (range = 36-233 kg, n = 400; Woods, unpublished data) at weaning. In these animals, after physical restraint, 100:1 ketamine: diazepam can be administered intravenously to effect based on a dose of 1 to 2 mg/kg ketamine. Diazepam is admistered first. Intramuscular administration can also be used but is less reliable.

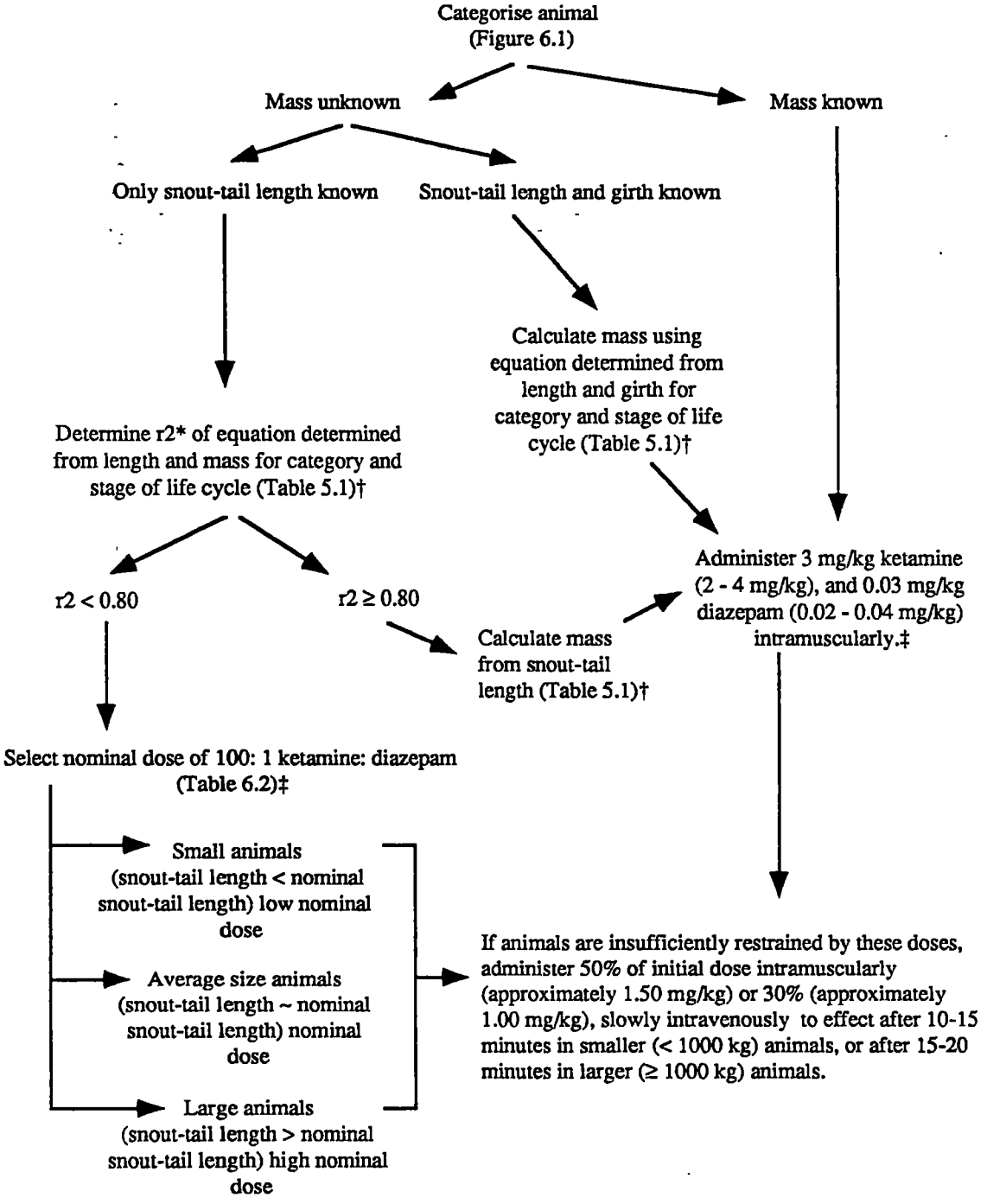

Figure 6.2. Procedure for estimating initial intramuscular dose of 100:1 ketamine: diazepam. Pups and weaners can be physically restrained and 100:1 ketamine: diazepam given intravenously to effect based on a dose of 1 - 2 mg/kg ketamine.
*r2 = coefficient of determination.
†If there is doubt as to the accuracy of the regression, consider use of nominal dose.
‡Dose of drug should be modified according to level of arousal, depressed or somnolent animals receiving doses at the lower range of those presented.

Table 6.3 Summary of expected normal responses (median, range), and those considered abnormal, when restraining large or small animals to level 3 - 4. (bpm = beats per minute).

Size of seal	Response	Heart rate (bpm)	Respiratory rate* (Breaths/ minute)	Common periods of apnoea†	Maximum level of restraint	Time to (minutes) maximum level of restraint	Time to (minutes) recovery (level 1 restraint)
Small (< 1000 kg)							
	Normal	60§ (34 - 104)	5§ (1 - 20)	< 10 minutesII or < 60s¶	3 - 4	10 (5 - 15)	< 60
	Abnormal	< 40 or > 80	< 4	> 10 minutesII or > 60s¶	< 3 or > 5	< 5 or > 15	> 60
Large (≥ 1000 kg)							
	Normal	48 (36 - 72)	4 (1 - 10)	< 20 minutesII or < 60s¶	3 - 4	20 (15 - 40)	< 90
	Abnormal	< 20 or > 60	< 2	> 20 minutesII or > 60s¶	< 2 or > 5	< 10 or > 40	> 90

*When breathing is stimulated using physical techniques (Table 13.4).

†Apnoea is a normal response to anaesthesia in elephant seals (Chapter 8 and 13). However, it can be treated, or prevented using physical techniques in the majority of animals. For this reason, in this study apnoea of >60s duration was considered abnormal It is especially important to prevent apnoea in bulls for which there are currently no effective techniques available for positive pressure ventilation.

§Heart and respiratory rates are higher in yearlings, heart rate = 100 bpm (range = 80-100 bpm), respiratory rate = 9 breaths/ minute (range = 6-20 breaths/ minute).

II If breathing is not stimulated using physical techniques (Table 13.4).

¶If breathing is stimulated using physical techniques (Table 13.4).

Notes

1. Normal rectal temperature is 37°C (range = 36 0 - 39.1°C), abnormal is > 38.5°C, or a rapid increase.
2. Respiration is characterised by quiet regular breathing interspersed with short periods of apnoea
3. There should be no shaking It should be treated if > level 2 shaking (see Appendix VI)
4. Mucus membranes remain dry, or with minimal salivation, and colour remains pink with capillary refill < 2-3 s Profuse, thick tacky saliva or white, grey or blue mucus membranes are abnormal.
5. There is some muscle tone, and palpebral response remains brisk.
6. Time and duration of maximum level restraint and time to recovery increase as size increases; heart and respiratory rates decrease.

Discussion

The fact that neither ketamine or diazepam dose level (mg/kg) decreased as mass increased was surprising given that this occurs in some other species (Sedgwick 1988) and indicates that a dose of 3 mg/kg ketamine combined with 0.03 mg/kg diazepam may be suitable for all sizes of animal.

In the absence of body mass data, the nominal doses of drug for each category of animal based on a dose of 3 mg/kg (2 - 4 mg/kg) ketamine and 0.03 mg/kg (0.02 - 0.04 mg/kg) diazepam presented in Table 6.1B are recommended. Drug dosage based on nominal doses of drug for each category of animal offers a safe and practical technique which avoids problems associated with the use of length-mass relationships and may be more appropriate in circumstances where these relationships are not close.

The nominal masses presented in this study (Table 6.2) could be used to determine doses of other drugs for each category of seal from known dose rates (mg/kg). However until experience is gained, or dose-response studies conducted, it may be preferable to use the lower range of known dose rates of these drugs to determine nominal doses in order not to overdose animals.

Drug dosage determination is based on knowledge of drug dose rates and the mass of the animal. However, in practice the final dose administered will have been adjusted according to a variety of factors including the desired response, the animal's level of arousal, health and response to injection; the assessment of which is largely determined by experience. There is no substitute for experience in drug dosage determination, however until more accurate techniques become available for estimating mass prior to drug administration, and dose-response studies are performed, the drug dosages presented here, coupled with the use of length-mass relationships and caution, could be used to determine 100:1 ketamine: diazepam dosage in southern elephant seals (Figure 6.2).

As apnoea or breathing difficulties were often induced by additional intravenous ketamine administration it may be advisable to administer drug slowly to effect and to be prepared to treat any ensuing apnoea.

The values describing the normal time course of restraint and the physiological parameters used in monitoring improve understanding of the expected response of southern elephant seals to 100:1 ketamine: diazepam restraint and allow deviations from normal responses to be recognised, thus aiding assessment of the anaesthetic episode and improving safety. Because responses to different anaesthetics vary (Chapter 8), values for these variables need to be determined for large and small animals for those drugs commonly used to chemically restraint southern elephant seals.

Size had a significant effect on response to 100:1 ketamine: diazepam. As the animal's size increased so did the time to reach the maximum level of restraint, its duration, and the time to recovery. These effects are probably due to pharmacokinetic differences associated with size and have important practical ramifications when anaesthetising larger animals. Most smaller animals became handleable within 10 to 15 min of initial drug administration, and most larger animals within 15 to 20 min (but this was as long as 45 min). Hence one should delay 15 to 20 min before administering additional drug to large animals to avoid risk of drug overdose compared with a 10 to 15 minute delay for smaller animals. The longer times to recovery also mean that associated problems, such as hyperthermia, pup abandonment, and attack by other seals, will be more pronounced as size increases. In particular, prolonged recovery of harem bulls can lead to attack by challenger bulls and disruption of the structure of the harem during the breeding season.

The decrease in heart rate and respiratory rate as mass increased was not unexpected (Gibbons *et al.* 1988, Sedgwick 1988, Sedgwick and Pokras 1988). The differences in heart rate and respiratory rate between small and large animals also has important ramifications during monitoring. For example, a respiratory rate of 2 breaths/ minute and heart rate of 40 bpm may be within normal limits for a 2000 kg bull but would be considered slow if seen in a 200 kg yearling.

As well as decreasing heart rate and respiratory rate, size appeared to have an effect on the effectiveness of respiratory exchange. Pv_{CO2} and pH were significantly lower at level 3 or 4 in larger animals compared with smaller animals, and Pv_{CO2} and HCO_3^- increased as mass increased. There are problems associated with interpretation of venous blood gas values (Haskins 1977) (Chapter 4) and the relationships were not close, so these data need to be interpreted with caution. However, Hammond and Elsner (1977) considered that resuscitation in large phocid seals could be complicated by the flexible thorax, evolved to allow collapse of the lungs during diving. It is possible that the increased Pv_{CO2} and HCO_3^- might reflect changes in ventilation and perfusion, possibly associated with changes in the rate and/ or depth of ventilation, or alveolar collapse or atelectasis due to an increase in compliance as the weight of the animal's thorax comes to bear on the lung parenchyma. If this is the case then larger animals may represent a greater anaesthetic risk than smaller animals and the use of positive pressure ventilation may be indicated. There are however problems associated with positive pressure ventilation in large animals and seals (Chapter 11) and maintenance on oxygen, or positioning these animals on their back and sides (a position which may aid resuscitation; Hammond and Elsner 1977) may be warranted until such time as arterial blood gas data, or techniques for positive pressure ventilation of large animals become available.

Chapter 7: Pharmacokinetics of intravenously administered ketamine

Introduction

Female southern elephant seals spend most of their life cycle at sea. However they come ashore twice each year, once to breed and once to moult. During these periods the animals do not feed and can lose up to 50% of their body mass, mostly as fat (Gales and Burton 1987 b). Woods *et al.* (1989) showed that there were differences in duration of sedation at different times of their life cycle and suggested that changes in sedative pharmacokinetics might be a contributing factor.

The dissociative anaesthetic ketamine is commonly combined with a sedative to chemically restrain southern elephant seals (Mitchell and Burton 1991). However there are no data available on the distribution of ketamine or other drugs in this species. The present study was to determine basic disposition kinetics* for ketamine in this species and investigate any differences with the animal's life cycle.

Materials and methods

Animals

Three groups of 5 mature female southern elephant seals of median 2.40 m snout-tail length (range = 2.20 - 2.95 m) were used in this study. They were: (1) pre-moulting cows, recently hauled ashore to moult; (2) post-moult cows, circa 20 days after pre-moult when the coat had been completely shed; and (3) pre-breeding cows within 1 day of giving birth (Woods *et al.* 1989).

Anaesthetic technique

To allow access for venipuncture, animals were sedated with a median dose of 5 mg/kg pethidine (range = 3 - 10 mg/kg) delivered intramuscularly by a remote injection technique (Ryding 1981). Once animals became heavily sedated a blood sampling needle (90mm, 18G spinal needle) was inserted into the extradural intravertebral vein approximately four lumbar vertebrae cranial of a line drawn between the wings of the ilium. A second drug administration spinal needle was placed approximately two vertebrae caudal to the first.

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* An introduction to pharmacokinetics - the disposition and fate of drugs in the body - can be found in Booth 1982 a.

Ketamine was then administered intravenously via the drug administration spinal needle over 60s. Pre-breeding and pre-moult animals received 500 mg ketamine and post-moult animals received 300 mg ketamine.

A stop-watch was started at the beginning of drug administration and blood samples were taken into 10 mL lithium heparin vacutainer blood collection tubes in duplicate at 2, 4, 6, 8, 10, 15, 20, 30, 45, 60, 75, 90, 120, 150 and 180 min post injection of ketamine. The blood sampling needle was kept patent by flushing with heparinised saline. Samples were centrifuged, plasma aspirated, mixed and distributed evenly into 2 nonsilanized serum tubes which were held frozen (below -21°C) until assayed.

To maintain sufficient sedation to allow sampling during the 180 min period, up to 5 incremental doses of from 1 - 3 mg/kg pethidine (in 10 - 20mL per dose) were administered as necessary intramuscularly via a needle placed in the epaxial musculature approximately 20 cm lateral to the dorsal midline. The total median dose of pethidine administered over the 180 min period for all animals was 9 mg/kg (range = 5 - 15 mg/kg).

Breathing was stimulated using physical techniques (Chapter 13) for the 180 min period. Mass was estimated using multiple regression relationships determined in Chapter 5.

Assay

The plasma was analysed for the presence of ketamine using a gas liquid chromatographic technique based upon that described by Waterman *et al.* (1987). Plasma was thawed at approximately 37°C and 1mL placed in a 20mL screw-top culture tube (Kimble, Illinois, USA), internal standard added (500 ng phencyclidine in 20 µL methanol), made alkaline by the addition of 1mL of pH 12.5 Na_2CO_3 buffer (20g $Na_2CO_3.10H_20$ in 100mL distilled water). This was rotated at 30 rpm for 30 min with 5mL benzene (Analytical reagent; May and Baker Australia, West Footscray, Victoria, Australia). The benzene was separated by centrifugation, aspirated and transferred into a 10mL screw-top centrifuge tube (Kimble, Illinois, USA). The benzene was rotated at 30 rpm for 10 min with 1mL 5M HCL then centrifuged, aspirated and discarded. One mL 5N NaOH, 1mL Na_2CO_3 buffer and 500µL toluene (Nanograde; Mallinckrodt, Clayton, Victoria, Australia), were then added to the HCL and the solution rotated at 30 rpm for 10 min, centrifuged, and the toluene transferred to a centrifuge tube where it was evaporated to approximately 20µL under a gentle stream of N_2. Samples of the toluene (1µL) were then injected on-column (15m, 0.53mm inside diameter, SE-30 wide-bore Econo-Cap capillary column with 1.2µm film thickness; Alltech Associates, Inc., 2051 Waukegan Road, Deerfield, IL 60015) in a Varian series 3700 gas chromatograph fitted with a thermionic specific detector. The carrier gas was helium at a flow rate of 3mL/min and the operating conditions were: injector temperature 230°C, detector temperature 300°C, and oven temperature 175°C for 2 min, increasing by 10°C/min to 300°C, held for 2 min.

The areas of the peaks obtained were measured by an integrator (Milton Roy CI-10B; SGE, Ringwood, Victoria, Australia) and the ratio of ketamine to internal standard was read off a calibration curve prepared by adding varying amounts of ketamine in 20 μL methanol to 1 mL elephant seal plasma to give final concentrations of 50, 100, 200, 250, 300, 400, 500, 1000, 2000, 3000, 4000, 5000, 6000 and 8000 ng/mL of ketamine/ 1mL plasma (500 ng of internal standard in 20 μL methanol was added to each). (An example chromatogram of ketamine and phencyclidine extracted from southern elephant seal plasma during this study is presented in Figure 7.1).

The calibration curve was linear over the range 10 ng/mL to 8 μg/mL ($r^2 = 0.999$, n = 18). Recovery and repeatability were determined by assaying 5 replicate extracts of high (5000 ng/mL) and low (100 ng/mL) concentrations of ketamine in plasma where:

$$\text{Recovery (\%)} = \frac{\text{(peak area ratio ketamine [extracted] / internal standard [not extracted])}}{\text{(peak area ratio ketamine [not extracted]/ internal standard [not extracted])}}$$

For extracted samples, high and low concentrations of ketamine (in 20 μL methanol) were added to 1 mL plasma and extracted (as above) into 500 μL toluene to which 500 ng internal standard (in 20 μL toluene) was then added and 1 μL of this solution injected on-column. For samples not extracted, high and low concentrations of ketamine (in 20 μL toluene) were added to 500 μL toluene, then 500 ng internal standard (in 20 μL toluene) was added and 1 μL of this solution injected on-column. Recovery (mean ± standard deviation) was greater than 70% (5000 ng/mL ketamine = 78±3%, n = 5; 100 ng/mL ketamine = 72±4%, n = 5).

Same day repeatability was determined by running 5 replicates of both high and low concentrations extracted on the same day. Same day coefficient of variation was 3% (5000 ng/mL ketamine = 3%, n = 5; 100 ng/mL ketamine = 3%, n = 5). Between day repeatability was determined by running a single replicate of high and low ketamine concentrations extracted on each day that a run was performed. The between day coefficient of variation was 8 - 9% (5000 ng/mL ketamine = 8%, n = 27; 100 ng/mL ketamine = 9%, n = 27).

Pharmacokinetic and statistical analyses

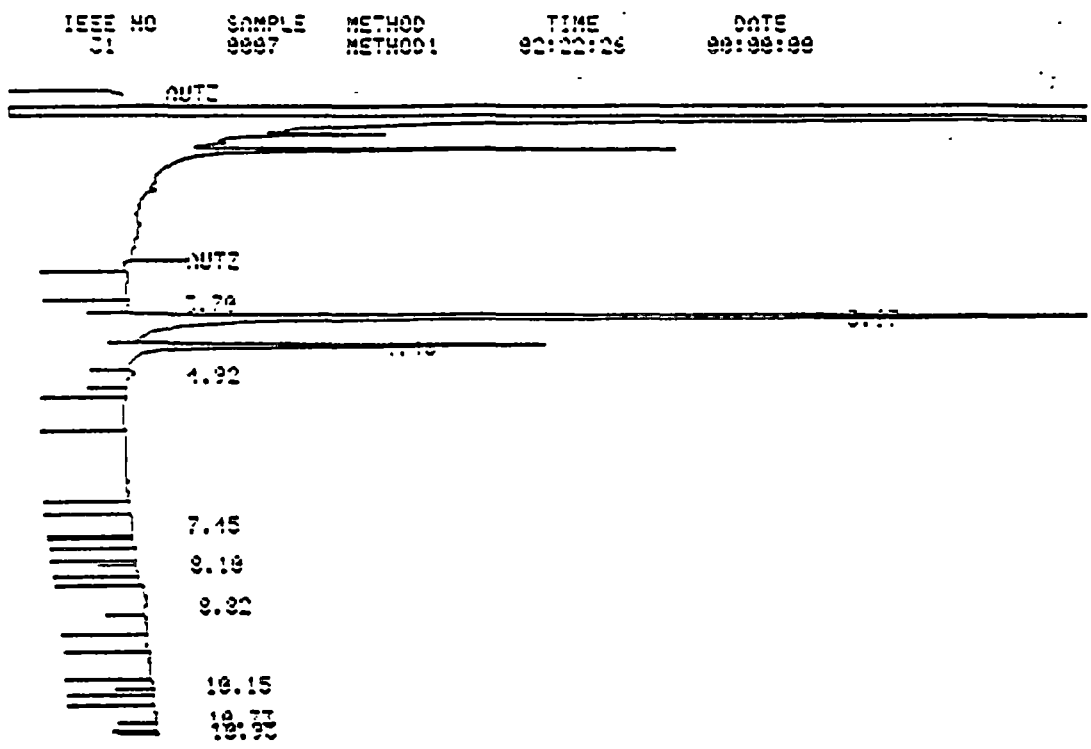
i. Determination of appropriate compartment model

The plasma concentration of ketamine was plotted against time and fitted to a two or three compartment model described by bi or triexponential equations of the form:

$$f(t) = a_1 e^{-b1t} + a_2 e^{-b2t}$$

or

$$f(t) = a_1 e^{-b1t} + a_2 e^{-b2t} + a_3 e^{-b3t}$$



DEFAULT 0
NORMALIZATION METHOD USING AREA

TIME	HEIGHT	AREA		%AREA
[illegible]	[illegible]	[illegible]	P	[illegible]
[illegible]	[illegible]	[illegible]	P	[illegible]
[illegible]	[illegible]	[illegible]		[illegible]
[illegible]	[illegible]	[illegible]	V	[illegible]
[illegible]	[illegible]	[illegible]		[illegible]
[illegible]	[illegible]	[illegible]		[illegible]
[illegible]	[illegible]	[illegible]	P	[illegible]
[illegible]	[illegible]	[illegible]		[illegible]
[illegible]	[illegible]	[illegible]		[illegible]

TOTAL 156493 100.0000000

Figure 7.1. An example chromatogram of ketamine and phencyclidine extracted from southern elephant seal plasma during this study. Ketamine eluted at approximately 4.0 minutes and phencyclidine at approximately 4.5 minutes. The concentration of ketamine in the plasma determined from this trace was approximately 1.86µg/mL. The sample was collected at 4 minutes.

respectively, using SigmaPlot® (Jandell Scientific, USA, 1992) transforms and curve fitting software package.

For each parameter dependencies were determined and used to ascertain the most appropriate model after the technique described in the manual (SigmaPlot® 1992) where dependence is defined as

$$\text{Dependence} = 1 - \frac{\text{(variance of the parameter, other parameters constant)}}{\text{(variance of the parameter, other parameters changing)}}$$

Parameter dependencies of greater than 0.99 were taken to indicate that the parameters were dependent and that the data had been "over-parameterized". (Parameters with dependencies near 1.0 are strongly dependent on one another. This may indicate that the equation(s) used are too complicated and "over-parameterized" - too many parameters are being used. A model with fewer parameters may be better (SigmaPlot® 1992).) The most appropriate model was thus considered to be that with the lowest number of curves containing dependent variables.

ii. Pharmacokinetic analysis

Based on the results of curve fitting (above) the plasma concentration of ketamine was plotted against time and fitted to a two compartment model, analogous to that presented above, and described by the biexponential equation:

$$C = Ae^{-\alpha t} + Be^{-\beta t}$$

where C = concentration in the plasma at time t (in min); A and B are zero time plasma drug concentration intercepts of the biphasic intravenous disposition curve (ng/mL) (A is based on the disposition and B the elimination phase); α and β are the overall distribution and elimination rate constants respectively, expressed in units of reciprocal time (min^{-1}); and e represents the base of the natural logarithm (Baggot 1982 a). These constants were calculated for each animal using a least squares, non-linear regression program (SigmaPlot® 1992) and the following kinetic parameters calculated using a method described by (Baggot 1982 a): $t_{1/2\alpha}$ (half life of distribution phase in min) = $0.693/\alpha$; $t_{1/2\beta}$ (half life of elimination phase in min) = $0.693/\beta$; V_d (apparent volume of distribution based on total area under under the plasma drug concentration time curve in mL/kg) = Dose/ $(A/\alpha + B/\beta)\beta$; and Cl_B (body clearance of a drug, which represents total body plasma clearance in $mLmin^{-1}kg^{-1}$) = $\beta.V_d$.

All values were expressed as the median and range. Data were analysed using the Kruskall-Wallis H test for three groups and the Mann-Whitney U test for two groups. Differences were considered significant when $P < 0.05$.

Results

General

Doses of ketamine administered to each of the 3 groups were not significantly different (median dose of ketamine = 1.1 mg/kg, range = 0.8 - 1.6 mg/kg, n = 15). The masses of animals were similar for pre-moult and pre-breeding animals (median = 398 kg, range = 322 - 577 kg, n = 10) which were significantly heavier than post-moult animals (median = 302 kg, range = 227 - 379 kg, n = 5; U = 4.0, df = 1, $P < 0.05$).

Associated with ketamine administration was an increase in level of chemical restraint from heavy sedation (level 3) to light immobilization (level 4; range 4 - 5; light to heavy immobilization) for approximately 10 - 15 min.

The respiratory pattern during chemical restraint was characterised by quiet, regular breathing interspersed with regular short (1 - 8 min) periods of apnoea. There were no prolonged periods of apnoea (> 10 min).

Determination of appropriate compartment model

The standard error and coefficient of variability for each parameter decreased with the addition of a third compartment to the model. However, the number of curves in which dependent variables occurred increased from 6 (in the 2 compartment model) to 12 (in the 3 compartment model).

Pharmacokinetic analysis

In most cases ketamine concentrations fell quickly in a biexponential fashion. When fitted to the 2 compartment model the initial distribution phase (α) had a median half life of 2.5 min (range = 1 - 11 min) while the slower elimination (β) phase had a median half life of 43 min (range = 17 - 108 min). A ketamine plasma concentration curve is shown in Figure 7.2. The calculated constants and other pharmacokinetic parameters are summarised in Table 7.1, and are characterised by great variability. It can be seen that the median apparent volume of distribution was 1475 mL kg^{-1} and median total body clearance was 33 mL $kg^{-1}min^{-1}$.

None of the pharmacokinetic constants or parameters were significantly different between the three groups (Kruskall-Wallis test; H range = 0.545 - 4.582, df = 2, $P > 0.05$), neither were they different when animals prior to fasting (pre-moult and pre-breeding) were compared with animals after fasting (post-moult) (Mann-Whitney U tests; U range = 12.0 - 22.0, $P > 0.05$).

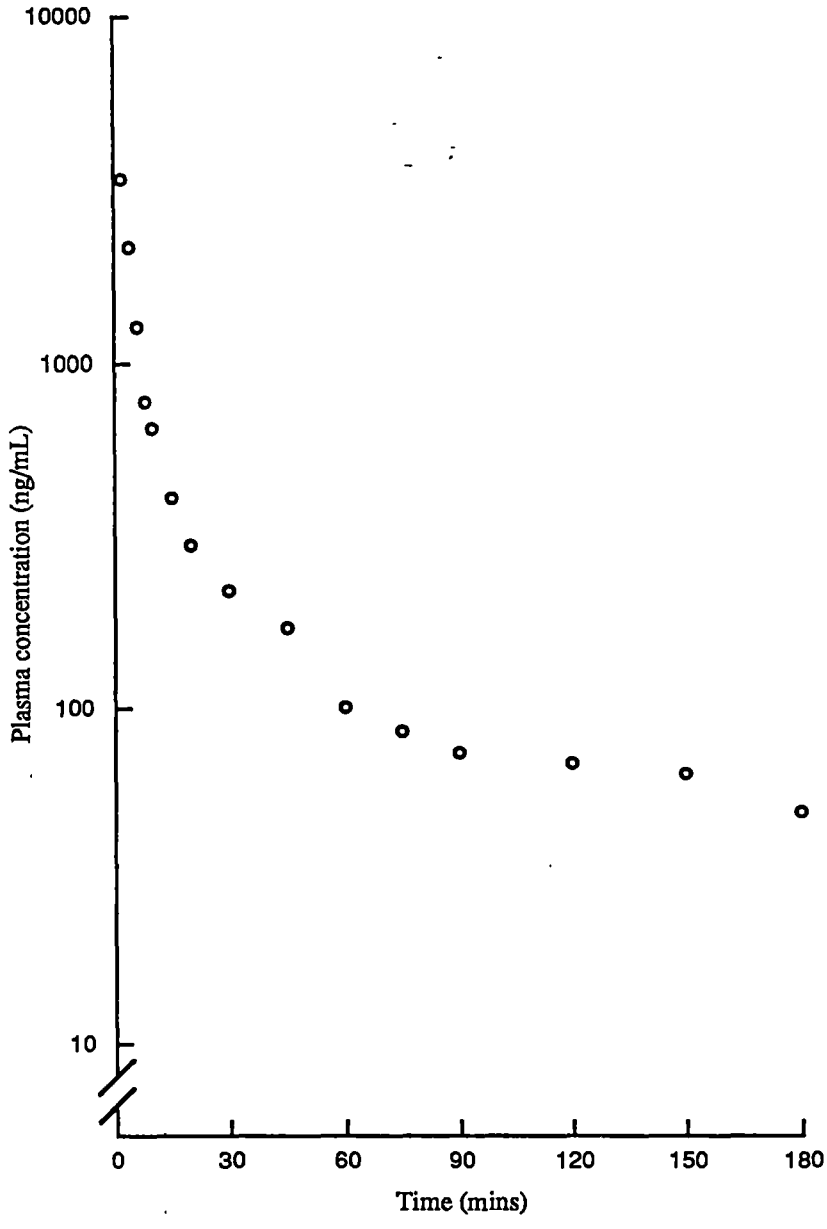


Figure 7.2. The plasma concentration of ketamine in a southern elephant seal after intravenous administration of drug at a dose rate of 1.55 mg/kg.

Table 7.1. Pharmacokinetic parameters (median, range) for ketamine fitted to a two compartment model in 3 groups of 5 female southern elephant seals. (A and B = zero time plasma drug concentration intercepts of the biphasic intravenous disposition curve, A is based on the disposition and B the elimination phase; α and β = the overall distribution and elimination rate constants respectively; $t_{1/2\alpha}$ = half life of distribution phase; $t_{1/2\beta}$ = half life of elimination phase; V_d = apparent volume of distribution; and Cl_B = body clearance of drug).

Group	Mass (kg)	A ($ng\ mL^{-1}$)	B ($ng\ mL^{-1}$)	α (min^{-1})	β (min^{-1})	$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (min)	V_d (mL kg-1)	Cl_B (mL min-1.kg-1)
Pre-breeding	517	4022.5	387	0.195	0.014	4	49	1500	33
	(360 - 577)	(572 - 8587)	(139 - 924)	(0.066 - 0.276)	(0.011 - 0.276)	(3 - 11)	(17 - 49)	(1493 - 5179)	(21 - 57)
Pre-moult	378	5326	584	0.463	0.024	1	28	1346	37
	(322 - 515)	(4325 - 8760)	(381 - 795)	(0.146 - 0.816)	(0.014 - 0.028)	(1 - 5)	(25 - 51)	(1032 - 2226)	(19 - 39)
Post-moult	302	3815	410	0.392	0.015	2	46	1804	25
	(227 - 379)	(1110 - 29447)	(174 - 906)	(0.155 - 0.61)	(0.006 - 0.040)	(1 - 4)	(19 - 108)	(830 - 9301)	(12 - 57)
All animals		5310	464	0.281	0.016	2.5	43	1475	33
		(572 - 29447)	(139 - 924)	(0.066 - 0.820)	(0.006 - 0.040)	(1 - 11)	(17 - 108)	(830 - 9301)	(12 - 57)

Discussion

Without the use of pethidine or other sedatives it would not have been possible to sample over the 180 min period used in this study. Other techniques for keeping the animals immobilized were considered such as the use of crush cages, however these were considered impractical given the nature of field work with the animals. Whilst sedated with pethidine animals appeared relaxed and unconcerned by sampling and the operators and it was a useful technique for maintaining heavy sedation.

The use of pethidine may, however, have affected the ketamine kinetics. Concurrent medication with other drugs has been shown to significantly affect ketamine kinetics in other species including reduction of volumes of distribution and clearance, and prolonging plasma half life (Waterman 1983 and 1984, Wood 1991). However there are no data available on the effect of pethidine on ketamine kinetics in any species. The duration of ketamine anaesthesia in monkeys is known to be prolonged by premedication with pethidine (McCarthy 1971) but it is not clear whether interactions between ketamine and other drugs are based on changes in the biodistribution of ketamine or if they reflect pharmacodynamic factors (White 1982). The effects of pethidine on the cardiovascular system of animals also appear to vary (Booth 1982 g), however in most species the cardiovascular system remains relatively stable after intramuscular administration (Anderson 1982). For these reasons it is difficult to predict the likely direction of effects of pethidine on ketamine kinetics in southern elephant seals. Use of physically restrained, previously catheterised animals would help resolve any possible effects but could create other problems such as increases in sympathetic tone. For this reason, use of conditioned, unrestrained captive animals in future studies might be preferred.

Despite the difficulties in comparing parameters with other studies this study does allow limited comparisons to be made between the groups of seals and shows that some pharmacokinetic data can be ascertained from wild, free-ranging animals.

As in most other species studied, plasma ketamine concentrations appeared to follow a biexponential decline (Figure 7.2). This observation, coupled with the increase in the frequency of occurrence of dependent variables (observed with the addition of a third compartment to the model), indicate that the 2 compartment model may be more appropriate than a 3 compartment model for describing ketamine kinetics in this species. However, dependent variables also occurred in 6 of the 15 curves generated using a 2 compartment model, indicating that in these cases a 1 compartment model may have been more appropriate (SigmaPlot® 1992). The range of values for parameters were also large and larger sample sizes, sampling over more prolonged periods and using differing ketamine dose levels are required to determine which model best describes ketamine kinetics in this species.

When fitted to a 2 compartment model, the median distribution phase ($t_{1/2\alpha}$) was only slightly more rapid in the southern elephant seals treated with pethidine (2.5 min) than in other animals to which ketamine had been administered either alone or combined with other drugs (2.8 to 17 min; Baggot and Blake 1976, Waterman and Livingston 1978, Kaka *et al.* 1979, Waterman *et al.* 1987, Clements and Nimmo 1981, Waterman 1984). The median elimination phase ($t_{1/2\beta}$; 43 min) was similar to that reported in studies in horses pre-medicated with xylazine (42 min; Kaka *et al.* 1979) and cats when ketamine was administered alone (43 min; Baggot and Blake 1976). The median apparent volume of distribution (1475 mL kg^{-1}) was not greatly different to values reported for horses (1625 - 2722 mL kg^{-1}; Kaka *et al.* 1979) and median total body clearance (33 mL $min^{-1}kg^{-1}$) was similar to that seen in horses (27 - 31 mL $min^{-1}kg^{-1}$; Kaka *et al.* 1979, Waterman *et al.* 1987).

As the initial steep decline in the plasma drug concentration is attributable mainly to the combined effects of intravascular mixing and distribution of the drug into tissues and organs of the body (Baggot 1982), it is likely that the relatively rapid distribution half life is due to the greater blood volume of southern elephant seals relative to other animals (207 mL/kg compared with 80 mL/kg in an adult human; Bryden and Lim 1969). There are no data available presenting cardiac output or perfusion of fat in southern elephant seals, however body fat content is higher compared with other animals (an estimated 26 - 37% in mature female southern elephant seals (M. A. Hindell; results of unpublished body composition studies) compared with between 5 and 20% for some other animals; Booth 1982a) and this could also account for, or contribute to, differences in initial distribution phase kinetics when compared with other species.

The difference in values were not great, however, and the wide range of values, coupled with the differing anaesthetic treatments, suggest that conclusions drawn from these data need to be viewed with caution. The wide range of values for the pharmacokinetic variables could be associated with changes in cardiac output, perfusion of tissues and fat associated with circulatory changes associated with apnoea, or thermoregulation, perhaps associated with warm days or moulting. Other factors such as differences in plasma protein and tissue binding at different times may also be involved. Plasma binding and tissue distribution studies, perhaps using tritium labelled ketamine (Sass and Salvatore 1977), at different times of the animals' life cycles would help clarify these points. Studies incorporating standardised sampling techniques and use of sedatives and larger sample sizes may be required to determine ketamine pharmacokinetic parameters more accurately in southern elephant seals.

The findings of this study indicate that intravenous kinetics of ketamine when administered with pethidine are similar for the 3 classes of seals. This was surprising given the large fluctuations in body mass of these animals at different times of the year and the differences in response to ketamine with xylazine between pre and post-fasting animals reported by Woods *et al.* (1989), and, if correct, supports Ryding's (1982) statement that the poorly vascularised blubber is not involved in the initial distribution of ketamine. However, the sample size is small, the range of data large and larger

samples sizes are required before a lack of difference can conclusively be shown. If, however, this finding is correct, it may be more appropriate to base intravenous doses of ketamine on lean rather than total body mass as has been suggested for humans (Wulfsohn 1972). Based on body composition data determined in pre-breeding and post-breeding females respectively, M. A. Hindell (unpublished data) considered total body fat to be 34% of total body mass in pre-fasting and 27% in post-fasting mature females. Slip *et al.* (1992) developed predictive relationships for total blubber and body fat in male southern elephant seals > 510 kg which could be used for estimation of lean body mass if total body mass was known in large animals (Appendix V).

The lack of difference in ketamine kinetics in the present study would suggest that the differences in durations of sedation reported by Woods *et al.* (1989) may be due to differences in absorption kinetics of the drugs, which were administered intramuscularly in their study, differences in xylazine kinetics, or other factors such as differences in sensitivity to drugs, at different times of the animals' life cycles.

The similarity in intravenous kinetics in the three classes of seals in the present study implies that to minimise variability in response, ketamine should be administered intravenously to southern elephant seals. However, Gales and Burton (1987a) reported that response to intravenous incremental doses of ketamine was less predictable than when drugs were administered intramuscularly in southern elephant seals though no data were presented. This is interesting given the advantages of rapid onset of effect and lower dose rate generally associated with intravenous drug administration. A comparison of intramuscular and intravenous dose response studies would clarify these points. However, until these studies are performed incremental doses of ketamine should be administered intravenously to female southern elephant seals to minimise variability in response and allow drugs to be given to effect. Chapter 6 offers guidelines to ketamine and diazepam administration in southern elephant seals.

Seals have developed a range of physiological changes associated with diving, including bradycardia and a decrease in cardiac output and peripheral circulation (Scholander 1940, Elsner *et al.* 1966, Elsner 1969, Kooyman *et al.* 1981, Butler and Jones 1982). Metabolism is also reduced in many tissues (Kooyman *et al.* 1981). Blood flow to the liver decreases and blood is stored in large venous sinuses (Harrison and Tomlinson 1958). These actions conserve oxygen and glucose for the heart and central nervous system (Kooyman 1968, Robin *et al.* 1981) and have been referred to as the "dive response" (Hubbard and Poulter 1968). On land southern elephant seals have been described as showing a rhythmic variation of breathing rate characterised by some minutes of eupnea followed by a period of apnoea of variable duration (Kenny 1979). There is evidence to suggest that, in the closely related northern elephant seal (*Mirounga angostirostris)*, this "sleep apnoea" has physiological aspects which are similar to those seen in freely diving seals (Castellini *et al.* 1986) and it has been theorised that

elephant seals (Gales and Burton 1987 a). It is thus possible that the wide range of values found for the pharmacokinetic parameters in this study may be associated with these "dive-response" changes.

Mitchell and Burton (1991) also observed that duration of chemical restraint in southern elephant seals increased as the total duration of apnoea increased. In other animals, redistribution of ketamine from the brain to other tissues is primarily responsible for termination of its hypnotic or anaesthetic effect (White *et al.* 1982). It is thus likely that apnoea and the associated changes in blood flow in anaesthetised seals affect ketamine pharmacokinetics, perhaps increasing half life by decreasing clearance as rate of diffusion from central to peripheral compartment and rate of elimination slow. Backhouse (1964) also suggested that when apnoeic, anaesthetised animals start breathing, anaesthetic laden blood held pooled in the hepatic sinus might rapidly be released into the heart-brain circulation increasing the level and/ or duration of restraint. Acid-base changes in blood and tissues during apnoea could also influence ketamine concentrations in the blood of apnoeic animals by affecting ionization. It is likely that apnoea will have marked effects on ketamine pharmacokinetics in southern elephant seals and these effects need to be examined. Prolonged apnoea was not seen during this study. Ketamine kinetics during apnoea are discussed in Chapter 13.

The bases of rational drug therapy are dose-response and pharmacokinetic studies yet, due to the impracticalities of field work there is a lack of this information. Drug dose-response and pharmacokinetic studies are indicated using different anaesthetic agents in other seal species. To gain the most benefit for comparative use methodology should be standardised and this may be most easily achieved using pre-catheterised, conditioned, captive animals.

Chapter 8: A comparison of some cyclohexamine based drug combinations

Introduction

There is limited information about the chemical restraint of elephant seals. The safest, most appropriate drug combinations are those which have the lowest fatality rate, and cause the least respiratory, central, cardiovascular and thermoregulatory depression. Suitable drug combinations also need to have predictable effects with rapid onset and termination of action, and be able to be used easily under field conditions by people without advanced training in anaesthetics.

The drug combinations, ketamine plus diazepam, ketamine plus xylazine, or tiletamine plus zolazepam have been administered previously to southern elephant seals (Gales and Burton 1987 a, Baker *et al.* 1988, Bester 1988, McCann *et al.* 1989, Woods *et al.* 1989, Baker *et al.* 1990, Mitchell and Burton 1991). Various ratios of ketamine and diazepam have been tried including 20:1 (Baker *et al.* 1988), 50:1 (McCann *et al.* 1989), 100:1 (Chapter 6) and others (Gales and Burton 1987 a). These reports did not show which of these ratios was most appropriate. However, all appeared relatively safe. Ketamine and xylazine use has been associated with fatalities and thermoregulatory problems (Vergani 1985, Woods *et al.* 1989). Ketamine and midazolam has not been used before, but midazolam has several potential advantages over diazepam as a sedative including water solubility, high lipophilicity at physiologic pH and rapid metabolism (Reves *et al.* 1985). The sedative effects of tiletamine and zolazepam were uncertain and therefore required further investigation (Baker *et al.* 1990, Mitchell and Burton 1991).

This study compared the effects of drug combinations commonly used for chemical restraint of southern elephant seals. The main aims were to gather basic information regarding the response of the animals to the different combinations, and to determine which were most useful for routine chemical restraint.

Materials and Methods

Five groups of 15 healthy, quiescent pre-moulting female southern elephant seals were used. The median snout-tail length (tip of the nose to the tip of the tail) was 2.52 m (range = 2.10 - 2.92 m). Each animal was given a single intramuscular dose of either ketamine and diazepam, ketamine and midazolam, ketamine and xylazine, or tiletamine and zolazepam. Ketamine and diazepam was given in two ratios to different groups, 100:1 ketamine to diazepam (100:1 ketamine: diazepam) and 50:1 ketamine to diazepam (50:1 ketamine: diazepam) (Table 8.1).

Table 8.1. Doses of drugs administered to 5 groups of female southern elephant seals and some response variables (median and range), and incidence of prolonged chemical restraint (time to recovery > 60 minutes).

Treatment	Number of animals	Drug dose (mg/kg)				Maximum level of chemical restraint	Time to recovery (min)	Number of episodes of prolonged restraint
		Anaesthetic (ketamine or tiletamine)		Sedative (diazepam, midazolam, xylazine or zolazepam)				
		Intended	Actual	Intended	Actual			
100:1 ketamine: diazepam	15	3.00	3.08 (2.35-3.93)	0.03	0.03 (0.02-0.35)	4 (3-5)	44 (18-79)	1
50:1 ketamine: diazepam	15	3.00	2.42 (1.97-4.78)	0.06	0.06 (0.05-0.12)	3 (1-6)	45 (24-90)	1
Ketamine and midazolam	15	3.00	2.66 (2.14-3.67)	0.02	0.02 (0.02-0.03)	4 (2-6)	47 (29-67)	2
Ketamine and xylazine	15	3.00	2.70 (2.06-3.14)	0.50	0.48 (0.22-0.54)	4 (2-6)	91.5 (40-191)	12
Tiletamine and zolazepam	15	0.50	0.48 (0.36-0.60)	0.50	0.48 (0.36-0.60)	3 (3-4)	52.5 (31-75)	6
			H = 11.45 df = 3 P < 0.01			H = 19.29 df = 4 P < 0.001	H = 24.82 df = 4 P < 0.001	Fisher's exact test df = 4 P < 0.001

There were no differences between groups in (a) time to maximum level of chemical restraint (median = 10 min, range = 1-30 min), (b) duration of maximum level of chemical restraint (median = 11 min, range = 1-60 min), and (c) time to first forward movement (median = 44 min, range = 18-81 min).

Drugs were administered by a remote injection technique after Ryding (1982). The doses were determined from past experience for ketamine and xylazine and 100:1 ketamine: diazepam (Woods *et al.* 1989, Mitchell and Burton 1991), tiletamine and zolazepam (Baker *et al.* 1990), and 50:1 ketamine: diazepam (McCann *et al.* 1989). The dose of midazolam in ketamine and midazolam was based on one administered to walruses (A. I. Webb, personal communication). Preliminary tests were made in a number of pre-moulting female seals to find doses to produce level 3 or 4 of chemical restraint (Table 4.2), which generally allowed intravenous access or weighing (level 4 only) with minimal depressant effects. These doses were then used as the basis for the drug trials, although the actual dose was adjusted for each animal, depending on its level of arousal and response to insertion of the needle, with the objective of achieving level 3 or 4 of chemical restraint.

The actual doses (mg/kg) administered were calculated for each animal (Table 8.1).

Beginning 10 min after administration of drugs each animal's reflexes and responses to stimuli were monitored and recorded at 5 min intervals. Level of chemical restraint was determined from this information and was graded from level 1 to level 8 (see Tables 4.1 and 4.2).

Blood (1 mL, heparinised) was also collected every 5 min from the extradural intravertebral vein. Samples were capped and stored in an ice water bath until assayed (within 60 min) and values for pH, $PvCO_2$, PvO_2, HCO_3^-, total CO_2, and base excess determined using the technique described in Chapter 3.

Blood sampling and assessment of reflexes and responses were continued until the animals had recovered (usually within 60 min). Since several (usually 4-5, range 1-16) assessment values were obtained at levels 3 or 4, the median was used as a single representative figure.

Table 8.1 shows the maximum level of chemical restraint, time to recovery (level 1) and prolonged chemical restraint (recovery in excess of 60 min). Animals were also assessed for shaking, apparent hyperthermia (moving into water and/ or flicking sand or water over the body), the duration and number of episodes of apnoea, evidence of upper respiratory tract obstruction (stertorous breathing, increased respiratory effort), tachypnoea ($\geq$ 10 breaths/ min for $\geq$ 10 min not following apnoea), excessive levels of restraint ($\geq$ level 6, where there was concern for the animal's welfare), and other notable occurrences.

Ambient dry bulb temperatures were recorded at the commencement of each episode of chemical restraint. When possible rectal temperatures were also recorded at 5 min intervals.

The normal respiratory pattern and heart rate were determined using untreated animals in two groups of 15, awake and dozing. Each animal was watched for 60 min recording heart rate and respiratory rate

every 2 min and any periods of apnoea. A single representative value for each variable for each animal was generated by taking the median of the recorded values.

Data were analysed non-parametrically using the Mann-Whitney and Kruskall-Wallis tests for comparison of groups. Differences in frequencies were tested with Chi square analysis, or Fisher's exact test when numbers were insufficiently large (Zar 1984). Differences were considered significant when $P < 0.05$.

Results

General

The median weight of animals was 402 kg (range = 250-633 kg, n = 75) and there were no significant differences between groups. However, the actual dose of ketamine differed between groups: the 100:1 ketamine: diazepam group received the highest dose (3.08 mg/kg), followed by ketamine and xylazine (2.70 mg/kg), ketamine and midazolam (2.66 mg/kg) and 50:1 ketamine: diazepam (2.42 mg/kg).

Fatality rate

There were no fatalities during this study.

Respiratory depression

Two patterns of respiration were apparent in undrugged seals. Awake animals breathed regularly for the 60 min period compared with dozing seals whose respiratory pattern was regular but punctuated by brief periods of apnoea. Respiratory rates were similar for both groups (5 breaths/ min, range = 3-8) and the longest period of apnoea was 8 min seen in a dozing seal.

Respiratory rate and periods of apnoea were similar in the groups to which drugs were administered (Table 8.2). Apnoea (> 1 min) occurred in 43 (57%) of the 75 animals. Most apnoeic animals underwent a single 5 min period (range = 2 - 58 min) of apnoea approximately 5 min (range = 0-19 min) after drug administration. The apnoea generally resolved on venipuncture, when animals were rolled over for weighing or as a result of stimuli during monitoring. Prolonged periods of apnoea (> 10 min) were only seen in three 100:1 ketamine: diazepam animals, lasting for 13, 44 and 45 min.

Many of the blood gas and pH variables (pH, HCO_3^-, total CO_2 and base excess) differed amongst the groups but PvO_2 and $PvCO_2$ did not (Table 8.3). The values usually remained relatively stable for the duration of chemical restraint. However, samples collected from two 100:1 ketamine: diazepam animals which were apnoeic for 44 and 45 min showed falls in PvO_2, pH and base excess, and increased $PvCO_2$ (Table 8.3).

Table 8.2. Descriptive statistics (median and range) for each response used in monitoring at level 3 or 4 of chemical restraint for 5 groups of southern elephant seals

Drug group	Response						
	Heart rate (beats/ min)	Head response	Palpebral response	Withdrawal response	Caudal flipper response	Muscle tone	Righting response
100:1 ketamine: diazepam	62	4	3	2	2	3	2
	(48-83)	(3-4)	(2-3)	(1-3)	(0-3)	(2-3)	(1-3)
50:1 ketamine: diazepam	58	3	3	1	1	2	2
	(48-72)	(2-3)	(2-3)	(0-3)	(0-2)	(1-3)	(0-3)
Ketamine and midazolam	54	3	3	1	2	2	2
	(46-63)	(2-4)	(3-3)	(1-2)	(1-3)	(1-3)	(1-3)
Ketamine and xylazine	50	4	2	1	1	1	1
	(37-61)	(3-4)	(2-3)	(0-3)	(0-3)	(0-3)	0-3)
Tiletamine and zolazepam	66	3	3	2	1	2	2
	(51-72)	(2-4)	(1-3)	(0-2)	(1-2)	(1-3)	(1-3)
All animals	59	3	3	1.5	1	2	2
	(37-83)	(2-4)	(1-3)	(0-3)	(0-3)	(0-3)	(0-3)
	H = 24.41	H = 25.90	H = 15.06	H = 12.75	H = 13.40	H = 29.22	H = 15.48
	df = 4	df = 4	df = 4	df = 4	df = 4	df = 4	df = 4
	P < 0.001	P < 0.001	P < 0.01	P < 0.05	P < 0.01	P < 0.001	P < 0.01

There were no significant differences between groups in (a) respiratory rate (median = 5 breaths/min, range = 1-10 breaths/min), (b) rectal temperature (median = 36.9°C, range = 35.0-38.0°C), and (c) capillary refill (median = <2 s, range = <1-<3s).

Table 8.3. Descriptive statistics (median and range) for each blood gas variable at level 3 or 4 of chemical restraint for 5 groups of seals, all seals, and those which underwent prolonged apnoea for 44 and 45 min.

Drug group	Blood gas variable					
	pH	PvO2 (mmHg)	PvCO2 (mmHg)	HCO3- (mmol/L)	Total CO2 (mmol/L)	Base excess (mmol/L)
100:1 ketamine: diazepam	7.31 (7.22-7.40)	43 (20-56)	50 (30-68)	26 (20-34)	28 (22-36)	-1 (-6-6)
50:1 ketamine: diazepam	7.35 (7.32-7.42)			27 (21-35)	29 (23-37)	0 (-3-7)
Ketamine and midazolam	7.34 (7.29-7.39)			28 (26-34)	30 (27-37)	2 (-1-5)
Ketamine and xylazine	7.31 (7.24-7.36)			26 (20-30)	28 (22-32)	-2 (-5-2)
Tiletamine and zolazepam	7.31 (7.27-7.36)			24 (16-29)	26 (17-31)	-3 (-8-2)
All animals	7.32 (7.22-7.42)	43 (20-56)	50 (30-68)	26 (16-35)	28 (17-37)	-1 (-8-7)
Animals apnoeic for 44 and 45 min*	7.11	8	79	27	30	-7
Comparison of drug groups	H = 15.99 df = 4 P < 0.05			H = 16.23 df = 4 P < 0.05	H = 15.43 df = 4 P < 0.05	H = 22.55 df = 4 P < 0.001

*Average values for two 100:1 ketamine: diazepam animals apnoeic for 44 and 45 min just prior to commencement of breathing.

Evidence of upper respiratory tract obstruction was seen in one 100:1 ketamine: diazepam and one 50:1 ketamine: diazepam animal; both recovered uneventfully. Tachypnea was seen in some (1 - 3) animals in all groups except ketamine and xylazine.

Central depression

Animals given ketamine and xylazine were more sedated and had better analgesia and skeletal muscle relaxation than those given other sedative combinations (Tables 8.1 and 8.2). However there were no differences in the proportions of animals in each group which reached the desired, and excessive, levels of chemical restraint.

Three animals in the tiletamine and zolazepam group showed responses during and after restraint that appeared to be hallucinatory behaviour. This behaviour was not seen in any other group. Animals would either proceed rapidly around the study site, apparently oblivious to operators, other seals and local topography, or lose "all inhibitions", licking, smelling and observing the operators, tussock and other seals in the area. All 3 animals were also hypersensitive to touch, with erection of all vibrissae, and had a dissociated, staring look to their eyes. These animals did not suffer from misadventure and appeared to have recovered fully within 2 h of initial drug administration.

Cardiovascular depression

Heart rates were similar for awake and dozing undrugged animals (median = 60 bpm, range = 48 - 84 bpm).

Heart rate, but not capillary refill, was significantly different between the drug groups. Heart rate was lowest for ketamine and xylazine (median = 50 bpm) and was also the easier to monitor in this group than in other groups.

Thermoregulatory depression

Few rectal temperatures were recorded since rectal thermometers could not be used at restraint levels < 3. Those rectal temperatures recorded remained relatively constant and were similar in all groups (Table 8.2). However, during recovery evidence of hyperthermia was seen in 8 animals given ketamine and xylazine and 1 animal given tiletamine and zolazepam, although their rectal temperatures could no longer be measured. The ambient temperature was similar for all groups (range = 5.7°C - 7.4°C). However, within the ketamine and xylazine group, the ambient temperature was significantly higher when episodes of hyperthermia were apparent (6.8°C) than for when they were not (5.1°C) ($U = 10.00$, $P < 0.05$).

Unwanted side effects

Shaking was seen in only one animal given ketamine and xylazine, but in 5-7 animals in all other groups. It usually involved a 10 min period (range = 1-33 min) of level 1 shaking (range = 1-3) approximately 15 min (range = 7-36 min) after drug administration.

Other side effects included what appeared to be increased lacrymation in two 100:1 ketamine: diazepam animals and one 50:1 ketamine: diazepam animal. Hypersensitivity to touch as an "intention tremor like" coarse, repeated, uncontrolled jerking of the head away from the touch was seen in two 100:1 ketamine: diazepam animals. Defecation occurred in one 100:1 ketamine: diazepam animal and 5 ketamine and xylazine animals.

Predictability of effects

The response to tiletamine and zolazepam appeared clinically to be more predictable than for the other groups. Assessment of head response in animals given ketamine and xylazine proved difficult since in some cases they appeared to be asleep but would suddenly rear up or lunge or attempt to bite when disturbed.

Onset and termination of action

The times for onset and duration of maximum level of chemical restraint were similar in all groups, but not the time to recovery (Table 8.1). Animals in the ketamine and xylazine group took approximately twice as long to recover as animals in other groups (median = 92 min v. 44-53 min). Although the time to first forward movement was not significantly different between the groups (median = 44 min), 50:1 ketamine: diazepam, ketamine and xylazine, and tiletamine and zolazepam animals moved forward at deeper levels of chemical restraint (level 2) than 100:1 ketamine: diazepam and ketamine and midazolam animals (level 1; Table 8.1). ketamine and midazolam and 50:1 ketamine: diazepam animals received a similar dose of ketamine, but the time to maximum level of chemical restraint was significantly shorter after ketamine and midazolam than after 50:1 ketamine: diazepam (7 v. 12 min; $U = 55.50$, $P < 0.05$).

The incidence of prolonged chemical restraint was significantly different between the groups (Fisher's exact test: $df = 4$, $P < 0.001$) and was most commonly seen in animals which received ketamine and xylazine (12 animals) or tiletamine and zolazepam (6 animals). In the other groups it was seen, at most, once.

Ease of use

The easiest drug to use was tiletamine and zolazepam. A single bottle contained 250 mg of each drug, which at an estimated dose of approximately 1 mL (100 mg) solution per 100 kg body weight made its preparation easier than for the other drug combinations.

Discussion

General

The doses of drugs administered in this study were either lower than those used in other studies (Gales and Burton 1987a, Bester 1988, Baker *et al.* 1988, Woods *et al.* 1989, Mitchell and Burton 1991) or similar (Baker *et al.* 1990), and were as low as could be administered to achieve the desired effect. This was reflected in the failure of some animals to reach levels 3 or 4 of chemical restraint. Without experience it would be difficult to achieve venipuncture in these animals, necessitating additional doses of drug.

The lack of difference between ketamine and diazepam and ketamine and xylazine reported by Gales and Burton (1987a) may be due to the larger doses they used.

Fatality rate

Other studies have reported fatality rates of 3% for ketamine and xylazine (n = 301), < 1% for ketamine and diazepam (n > 83) (Mitchell and Burton 1991), and 1% for tiletamine and zolazepam (n = 200) (Baker *et al.* 1990, Mitchell and Burton 1991). However, these fatality rates should be interpreted with caution, since dose rates were not standardised and their administration was on a trial and error basis by different operators.

Respiratory depression

The blood gas and pH values presented suggest that some degree of respiratory depression may have been induced in each of the groups. Although there are difficulties associated with interpretation of venous blood gas values (Haskins 1977), the values gave useful information which was easily and quickly gathered in situations where arterial sampling was impractical. However, measuring oxygen saturation of arterial haemoglobin, perhaps of the tongue, using non-invasive pulse oximetry could be a more useful technique for assessment of cardiopulmonary function in these animals.

The prolonged apnoea and upper respiratory tract obstruction associated with ketamine and diazepam use was of concern, and unexpected given the generally good results reported by Baker *et al.* (1988). If the venous blood gas values correlate well with central arterial values, then the fall in Pv_{O2}, pH, and base excess and the rise in Pv_{CO2} seen in these animals may be disadvantageous, perhaps causing sympathetic stimulation, acidosis and central nervous system depression (Eicker 1986).

Other investigators have used higher doses of diazepam or ketamine with few problems (Gales and Burton 1987a, Baker *et al.* 1988). In humans diazepam is usually administered with ketamine to decrease the likelihood of emergence delirium. Perhaps stress caused by hallucinations or prolonged induction can lead to apnoea in animals which receive too little diazepam. If this is the case then higher doses or ratios of diazepam:ketamine may be more appropriate.

There is currently interest in whether apnoea should be considered an abnormal occurrence whilst chemically restraining seals. The respiratory pattern of chemically restrained seals in this study tended to be similar to that seen in the dozing control animals: regular respiration punctuated by brief periods of apnoea. However, no periods of more than 10 min were seen in dozing control animals suggesting that prolonged apnoea in chemically restrained southern elephant seals is likely to be a drug side effect.

Central depression

All drug combinations produced a degree of central nervous system depression which was appropriate for the procedures being performed. The slower reflexes and responses after ketamine and xylazine indicate either greater central nervous system depression, greater skeletal muscle relaxation or more profound analgesia. All of these effects have been described for xylazine in other species (Booth 1982 c). The greater skeletal muscle relaxation seen with ketamine and xylazine compared with, for instance, 100:1 ketamine: diazepam may be advantageous for surgical purposes. Although surgical anaesthesia is rarely required during field work, the improved relaxation did aid in handling animals.

The hallucinatory behaviour seen with tiletamine and zolazepam has not been reported in seals and, though not considered a problem in this study, could lead to misadventure or increase the chances of pup abandonment. However pup abandonment was not seen when the drugs were used to chemically restrain cows with pups by Baker *et al.* (1990) who indicated that the rate of abandonment was higher for animals to which ketamine and diazepam had been administered.

Cardiovascular depression

The lower heart rate recorded for ketamine and xylazine did not appear to compromise the animals and may not represent a problem as long as cardiac output and peripheral perfusion are maintained. However xylazine is known to cause cardiovascular depression in other species (Muir and Piper 1977, Booth 1982 c, Lumb and Jones 1984 b) and Woods *et al.* (1989) suggested that the use of ketamine and xylazine might predispose animals to enter a dive response. For these reasons drug combinations other than ketamine and xylazine were preferred as causing less cardiovascular depression.

The lower heart rate in ketamine and xylazine animals, coupled with what appeared to be greater excursions of the body wall associated with each beat, did, however, have the distinct advantage of making heart rate assessment easier than for other groups. This was probably also facilitated by the increased muscle relaxation and low incidence of shaking seen with ketamine and xylazine relative to the other groups.

Thermoregulatory depression

As ambient temperatures did not vary significantly the high incidence of apparent hyperthermia after ketamine and xylazine is likely to be a drug effect, as previously suggested (Vergani 1985, Woods *et al.* 1989). As hyperthermia was more prevalent at temperatures over 5°C it might be expedient not to use ketamine and xylazine unless the temperature is below 5°C or cooling procedures are used. However, solar radiation and wind may be more important than ambient temperature in determining heat load and for this reason cooling should be instigated if there is doubt as to the animal's thermoregulatory status.

The single episode of apparent hyperthermia for tiletamine and zolazepam was also of concern, perhaps more so than for ketamine and xylazine since unexpected. Hyperthermia has not been linked to tiletamine and zolazepam use in other species, but hypothermia has been reported in northern sea lions (*Eumetopias jubatus*) (Loughlin and Spraker 1989), and problems of prolonged recovery in non-pinniped species (Eads 1976, Boever *et al.* 1977). It is possible that the "sand-flicking" which was taken to be a sign of hyperthermia may have been associated with other stress, such as hypothermia or prolonged recovery (Laws 1956 b).

Unwanted side effects

The shaking associated with administration of diazepam and midazolam in the ketamine and diazepam and ketamine and midazolam combinations could indicate that the dose of these benzodiazepines was too low. Others investigators have used higher doses of diazepam (0.1 mg/kg) in ketamine and diazepam (Gales and Burton 1987a) or a decreased ratio of ketamine to diazepam (20:1) (Baker *et al.* 1988), without reporting shaking, and these doses are probably more routinely useful than the ketamine and diazepam doses used in this study. Similarly, the dose of midazolam could also be increased, however caution would need to be exercised given midazolam's greater potency relative to diazepam in other animals (Reves *et al.* 1985).

Predictability of effects

The response to tiletamine and zolazepam was more predictable than that for ketamine and diazepam and ketamine and xylazine, supporting the finding of Baker *et al.* (1990). However the response to the other drug combinations was also generally predictable, perhaps because of the equivalent doses and use of similar animals, thus minimising variability.

The aggression associated with the unpredictable head response seen in some animals with ketamine and xylazine was a disadvantage, but once recognised, precautions could be taken to avoid being bitten. Though unexpected defensive reactions have been associated with xylazine use in other animals (Booth 1982 c), there are no reports in the literature which describe this aggressive behaviour in seals. However, B. Joseph (personal communication) observed that captive pinnipeds sedated with

xylazine appeared very depressed, but were capable of reacting very aggressively. Possibly the benzodiazepine components of the other drug combinations also had a more calming effect (Booth 1982 d).

The inability to predict prolonged periods of apnoea or upper respiratory tract obstruction prior to ketamine and diazepam administration was also a disadvantage.

Onset and termination of action

Drug combinations having rapid onset and termination of actions are preferred, to minimise problems such as pup abandonment associated with prolonged chemical restraint (Bester 1988) and attack by other seals or sympatric species, and to facilitate efficient work with the animals. All the combinations fulfilled these criteria except for ketamine and xylazine for which time to recovery was approximately twice that of other groups. However ketamine and xylazine treated animals moved away at similar times to other groups which received ketamine indicating that the xylazine was probably responsible for the prolonged recovery. It has been reported that larger dosages of xylazine increase the duration rather than the degree of sedation (Lumb and Jones 1984 b). Possibly the dose of xylazine in ketamine and xylazine could be reduced, with a corresponding decrease in time to recovery. However, in some cases prolonged recovery could be an advantage, for instance when a data-logging apparatus is being glued to the animal.

Midazolam, which has a faster and briefer action than diazepam in other species (Reves *et al.* 1985), may be preferable as a sedative when combined with ketamine in this species.

Ease of use

Tiletamine with zolazepam was the easiest combination to use since the dose calculations were simple and volumes were small, enabling doses to be administered quickly. This would be of greater advantage still if drug had to be delivered by hand-held, extension, or projectile syringes rather than by a modification of Ryding's (1982) remote injection technique. However, smaller volumes of concentrated drug also have the potential to increase the likelihood of relative or absolute drug overdosage.

The inability to vary the ratio of components in the commercially available preparations of tiletamine and zolazepam, given their different rates of metabolism and elimination (Schobert 1987), represents a minor, but perhaps important theoretical disadvantage of its use, for example when animals need to be sedated for prolonged periods of time and receive repeated administrations of tiletamine and zolazepam.

Safety

Safety margins for drugs are difficult to determine from this study which used a narrow range of doses. The doses of benzodiazepine in the ketamine and diazepam and ketamine and midazolam groups could probably be increased with more safety than the xylazine in the ketamine and xylazine group because (1) they were low and (2) higher doses of xylazine have depressant effects on the thermoregulatory and cardiovascular systems (Lumb and Jones 1984 b).

Tiletamine with zolazepam was relatively safe at the doses used, as found by Baker *et al.* (1990). Administration of approximately twice this dose was associated with complications and fatalities (Mitchell and Burton 1991). This suggests that the therapeutic index of tiletamine and zolazepam in this species is relatively low, indicating caution, given the difficulties in estimating the mass of animals prior to drug administration. However, this study and those of Baker *et al.* (1990) and Mitchell and Burton (1991), give information on the range of doses that can safely be used: 0.25 - 0.5 mg/kg of each drug being relatively safe, > 1 mg/kg of each being associated with complications and fatalities. The prolonged apnoea and apparent upper respiratory tract problems associated with ketamine and diazepam use and the prolonged chemical restraint and apparent hyperthermia associated with ketamine and xylazine use indicate that ketamine and midazolam and tiletamine and zolazepam may be the "safer" of the drug combinations.

Most appropriate drug combinations

The drug combinations trialed could all be used safely to chemically restrain mature, pre-moulting female southern elephant seals when coupled with an accurate system of monitoring and early recognition and treatment of complications. However, for people without experience in anaesthesia of elephant seals tiletamine and zolazepam offered advantages in preparation, predictability and dosage. The conficting reports of the safety of tiletamine and zolazepam are probably due to drug over-dose during initial trials when safe doses were not known. These initial reports indicate that its safety margin may not be large so care should be taken in estimating masses of animals prior to drug administration. Though not yet trialed in southern elephant seals, use of antagonists could decrease this problem and improve control when using tiletamine and zolazepam or other drug combinations.

It is likely that the doses of drugs used in this study could be used to chemically restrain other classes of southern elephant seals, but variability in response to drugs at different times of the animal's life cycle and size related effects on dosage would need to be considered.

Other than for ketamine and diazepam, the study did not examine changing the ratio of the other drugs used, and there is scope for further work on this.

Chapter 9: Use of midazolam, pethidine, ketamine and thiopentone

Introduction

Cyclohexamine-based drug combinations are commonly used to restrain southern elephant seals (*Mirounga leonina*) (Gales 1989, Baker *et al.* 1990, Mitchell and Burton 1991); however, they have the disadvantage that the cyclohexamine component cannot be completely antagonised. The availability of narcotic antagonists offers potential advantages in improving the control and safety of narcotic-based anaesthesia.

Narcotic-based drug combinations based on pethidine have been used successfully to restrain northern elephant seals (*Mirounga angostirostris*), common seals (*Phoca vitulina*) and walrus (*Odobenus rosmarus*) (Cornell and Antrim 1987, Joseph and Cornell 1988, A. I. Webb personal communication 1989). Intramuscular pethidine was used either alone (0.11 - 0.45 mg/kg), or combined with midazolam (0.04 mg/kg and 2 mg/kg pethidine) as a sedative and to allow intravenous administration of the barbiturates thiamylal (0.74 mg/kg) or thiopentone (3 - 5 mg/kg) for induction of anaesthesia and intubation. These doses of pethidine, and midazolam and pethidine were insufficient to allow intravenous access without physical restraint. Additional doses of thiamylal or isoflurane were used to maintain anaesthesia, and doxapram and/ or naloxone (0.004 - 0.009 mg/kg) were used to speed recovery.

In the present study the usefulness of sedation with a narcotic-based drug combination was examined in southern elephant seals. There are no reports in the literature of narcotic-based drug combinations having been administered to these animals.

The aim was to develop a safe, reversible, versatile technique for chemical restraint, which could also be used to prepare animals for anaesthesia with other agents.

Materials and Methods

Animals and drug administration

The animals used were quiescent pre-moulting female southern elephant seals of median snout-tail length 2.58 m (range = 2.00 - 2.90 m). Three drug treatments, giving different levels of anaesthesia, were studied together with two narcotic antagonists (Table 9.1). Each animal was heavily sedated with 15 mg midazolam combined with pethidine (750 or 1500 mg) and 1.3 mg atropine delivered intramuscularly by a remote injection technique (Ryding 1982). Subsequent doses of saline or other

Table 9.1. Median (range) of values for response variables in 3 groups of mature female southern elephant seals chemically restrained with midazolam and pethidine based drug combinations. Where appropriate, values have been grouped for clarity. The midazolam and pethidine doses are given in mg, see text for full drug dosages. The median level of restraint for all groups for the period 10 to 20 minutes was 3 (range = 1 -3, n = 32). (min = minutes.)

Part	Drug group	Maximum level of chemical restraint*	Time to maximum level of chemical restraint (min)	Duration of maximum level of chemical restraint (min)	Duration of anaesthetic effect (min)‡	Time to recovery (light sedation) (min)	n
A	15:750 midazolam, pethidine and saline	3 (1 - 3)	7 (5 - 15)	43 (15 - 64)		88 (73 - 164)	6
	15:750 midazolam, pethidine and naloxone	3† (2 - 3)	8† (4 - 12)	15† (10 - 20)	(Not reached)	24† (21 - 26)	2
	15:1500 midazolam, pethidine and naltrexone	3 (3 - 3)	12 (5 -19)	13 (5 - 26)		30 (24 - 90)	5
B	15:1500 midazolam, pethidine, thiopentone and saline	4.5 (3 - 7)	22 (21 - 24)	6 (1 - 20)	13 (4-26)	113 (42 - 145)	6
	15:1500 midazolam, pethidine, thiopentone and naloxone						4
	15:1500 midazolam, pethidine, ketamine and saline	4 (3 - 6)	24 (22 - 26)	4 (3 - 6)	12 (10 - 14)	128 (110 - 170)	5
	15:1500 midazolam, pethidine, ketamine and naloxone	4	23	4	20	90	1
C	15:1500 midazolam, pethidine and ketamine for maintenance	5 (4 - 6)	22 (21 - 22)	62§ (46 - 79)		155 (110 - 205)	3

*1 = Light sedation, 2 = moderate sedation, 3 = heavy sedation, 4 = light immobilization, 5 = heavy immobilization, 6 = light anaesthesia, 7 = moderate anaesthesia.

†Average values.

‡Time taken to return to their previous level of chemical restraint.

§Median (range) of times for which level of chemical restraint ≥ 4 was maintained.

drugs were administered by intravenous injection (over 60 s) into the extradural intravertebral vein. All drug doses and responses were recorded.

There were three parts to the study.

A. Midazolam combined with pethidine for sedation

The two doses of pethidine combined with 15 mg midazolam were assessed for producing sedation and allowing intravenous access: 750 mg pethidine (15:750 midazolam and pethidine) and 1500 mg pethidine (15:1500 midazolam and pethidine). Twenty min after 15:750 midazolam and pethidine animals received either 40 mg naloxone (15:750 midazolam, pethidine and naloxone) or saline (15:750 midazolam, pethidine and saline); and after 15:1500 midazolam and pethidine, a dose of 1 mg naltrexone (15:1500 midazolam, pethidine and naltrexone). The volumes of antagonist and saline were similar and were warmed to approximately 37°C. Unless otherwise stated, all times are given from administration of midazolam and pethidine.

B. Midazolam and pethidine combined with thiopentone or ketamine to allow intubation

At approximately 20 min after 15:1500 midazolam and pethidine, a dose of 500 to 2000 mg of either thiopentone (15:1500 midazolam, pethidine and thiopentone) or ketamine (15:1500 midazolam, pethidine and ketamine) was administered, with the intention of inducing light to heavy immobilization (Table 9.1, footnote) to allow intubation. The range of doses was to accommodate a range of sizes and demeanours of animals. Thiopentone or ketamine were administered before 20 min if animals were insufficiently restrained by 15:1500 midazolam and pethidine and there was concern that they would become unapproachable. Lower doses were used in somnolent animals, higher doses in excited or aggressive animals. If necessary, additional doses of 500 mg thiopentone or ketamine were administered until adequate chemical restraint was achieved.

Approximately 30 min after midazolam and pethidine, either saline or naloxone (40 mg) was administered. Naloxone was administered early if administration of thiopentone or ketamine had caused dangerously deep anaesthesia.

C. Midazolam and pethidine combined with ketamine for prolonged immobilization

Three animals were used to assess the usefulness of ketamine for maintenance of anaesthesia after induction of level 4 - 5 restraint with 15:1500 midazolam, pethidine and ketamine. An area over the plantar venous arch was infiltrated with local anaesthetic and arterial cut-down performed. A level of chemical restraint which allowed surgery to proceed was maintained for approximately 60 min by administration of 500 mg boluses of ketamine.

Animals in Parts B and C were intubated after thiopentone or ketamine administration (usually between 20 and 26 min). Intubation was performed blind using a 24 mm outside diameter endotracheal tube (not inflated).

Monitoring responses

A standard monitoring sheet was completed for each episode of chemical restraint. Blood samples were taken from the extradural intravertebral vein for blood gas and pH measurements, and heart rate, respiratory rate and level of chemical restraint were monitored (Table 9.2). For each animal the maximum level of chemical restraint and its time of onset, duration and recovery to light sedation were also recorded. After thiopentone or ketamine administration, the duration of anaesthetic effect was taken as the time for animals to return to their previous level of chemical restraint (usually level 3).

In order to compare the effects of different drug treatments, single values for heart rate, respiratory rate, pH, blood gas values and level of chemical restraint for each animal were calculated as follows. (1) The extent of sedation after midazolam and pethidine was measured by the median of values for each of these variables at 10, 15 and 20 min. (2) The effect of thiopentone or ketamine was measured by the values recorded immediately prior to intubation. (3) The response to saline or narcotic antagonist was determined by the median of three values recorded during the 10 min following their administration.

Data were analysed using the Mann-Whitney U test for comparison of groups, Spearman rank order correlation (r_S) for relationships between variables, and Fisher's exact test for differences in frequencies. Differences were considered significant when $P < 0.05$.

Results

General

The median mass of all 32 animals was 375 kg (range = 225 - 634 kg) and the median dose of midazolam 0.04 mg/kg (range = 0.02 - 0.07 mg/kg). The 750 mg dose of pethidine gave a median dose of 2.25 mg/kg (range = 1.17 - 2.61 mg/kg, n = 8) and the 1500 mg dose, 3.55 mg/kg (range = 2.67 - 6.68 mg/kg, n = 24). The median dose of naloxone was 0.094 mg/kg (range = 0.086-0.104 mg/kg, n = 11). This gave a ratio of pethidine: naloxone of approximately 20:1 for 750 mg groups and 40:1 for 1500 mg groups. Naltrexone was administered to 5 animals sedated with 15:1500 midazolam and pethidine, at a median dose of 0.002 mg/kg (range = 0.001-0.003 mg/kg) giving a ratio of pethidine: naltrexone of approximately 1500:1.

Table 9.2. Level of chemical restraint, heart rate, respiratory rate, blood gas and pH values for 10 min periods before and after each treatment with saline, anaesthetic or antagonist for southern elephant seals chemically restrained with midazolam and pethidine based drug combinations. Where appropriate, values have been grouped for clarity. Values recorded for animals in Part C remained relatively stable for the 60 min period, however respiratory rate occassionally fell after ketamine administration.

Part	Drug group and 10 min time period monitored	Level of chemical restaint*	Heart rate (beats/ min)	Respiratory rate (breaths/min)	pH	PvO2 (mmHg)	PvCO2 (mmHg)	HCO3- (mmol/L)	Total CO2 (mmol/L)	Base excess (mmol/L)	n
A,B and C	All animals which received midazolam and pethidine (before saline, anaesthetic or antagonist administration)	3 (1-3)	60 (42-84)	6 (2-18)	7.26 (7.21-7.34)	38 (16-59)	52 (38-67)	25 (15-30)	27 (16-33)	-4 (-11-1)	32
A	15:750 midazolam, pethidine and saline (after saline)	3 (3-3)	60 (48-76)	6 (2-15)	7.27 (7.22-7.34)	38 (30-43)	57 (49-68)	27 (21-34)	29 (23-36)	-2 (-7-3)	6
	15:750 midazolam, pethidine and naloxone (after naloxone)	1† (1-1)	74† (68-80)	6† (4-8)	7.28† (7.24-7.32)	50† (49-52)	49† (49-49)	23† (21-25)	24† (23-26)	-4† (-7-2)	2
	15:1500 midazolam, pethidine and naltrexone (after naltrexone)	1 (1-3)	72 (69-84)	8 (4-15)	7.26 (7.22-7.29)	49 (38-56)	61 (53-66)	27 (25-31)	29 (27-33)	-3 (-4-1)	5
B	15:1500 midazolam, pethidine, thiopentone and saline (after saline)	4.5 (3-7)	62 (48-80)	3 (0-7)	7.30 (7.21-7.32)	39 (23-52)	56 (50-58)	28 (21-30)	30 (24-32)	-1 (-8-2)	6
	15:1500 midazolam, pethidine, thiopentone and naloxone (after naloxone)				7.22 (7.15-7.28)			23 (23-25)	25 (24-27)	-7 (-7--5)	4
					U = 2.0 P< 0.05			U = 1.0 P< 0.05	U = 1.0 P< 0.05	U = 2.0 P< 0.05	
	15:1500 midazolam, pethidine, ketamine and saline (after saline)	3 (3-3)	61 (56-72)	6 (0-7)	7.24 (7.21-7.24)	36 (13-49)	52 (50-69)	20 (17-30)	22 (19-33)	-7 (-10--1)	5
	15:1500 midazolam, pethidine, ketamine and naloxone (after naloxone)	2	66	6	7.31	58	47	24	26	-2	1
	15:1500 midazolam, pethidine, thiopentone or 15:1500 midazolam, pethidine, ketamine (after thiopentone or ketamine for both groups which received it)‡	4 (3-7)	62 (48-80)	3.5 (0-7)	7.26 (7.19-7.34)	38 (20-60)	54 (47-58)	23 (18-29)	25 (20-32)	-5 (-9-1)	16

*See Footnote Table 9.1.

†Average values.

‡For the period after thiopentone or ketamine administration but prior to intubation.

A. Midazolam combined with pethidine (Parts A, B and C)

The 15:750 midazolam and pethidine dose allowed intravenous access only in quiet, relaxed or somnolent animals and usually produced about 45 min of level 3 chemical restraint. The animals took about 10 min to reach this level (range = 5 - 15 min) and animals had usually recovered by 90 min (Table 9.1). Intravenous access was easier after the 1500 mg pethidine dose, and this was subsequently used in Parts B and C.

Time to recovery was not examined after the 1500 mg pethidine dose. Due to low drug stocks and supply difficulties at Macquarie Island each animal which received 15:1500 midazolam and pethidine was also treated with other drugs (Table 9.2). However, the 15:1500 midazolam and pethidine dose produced profound, long lasting sedation, muscle relaxation, and analgesia in most animals. Animals entered a sleep-like state from which they could be roused only with difficulty. The respiratory pattern was characterised by intermittent short periods of apnoea and/ or panting or shallow respiration. Monitoring was sometimes complicated by the opening and closing of the nares without apparent inspiration or expiration. This apnoea or shallow respiration could be treated by rousing the animals with a gentle tap on the nose or manipulation of the muzzle. After 1500 mg pethidine doses animals also appeared to lose all fear, and became oblivious to people and animals around them; two animals allowed themselves to be rolled over onto a sling for weighing.

Reversal of sedation with antagonists

Naloxone and naltrexone decreased both the duration of maximum chemical restraint and the time to recovery to about one third of control values (Table 9.1). The level of chemical restraint during the 10 minute period after antagonists was lower (level 1) than after saline administration (level 3) (Table 9.2). Median heart rate and Pv_{O2} were higher after naloxone and naltrexone (heart rate = 72 - 74 bpm, Pv_{O2} = 49 - 50 mmHg) than after saline (heart rate = 60 beats/ min, Pv_{O2} = 38 mmHg) (Table 9.2).

Evidence of relapse was seen in one animal which received naltrexone and recovered by 26 min only to relapse to level 3 at 58 min. The animal returned to level 1 by 80 min. Three other animals became quieter between 20 and 40 min after antagonist but without a change in level of chemical restraint.

B. Midazolam and pethidine combined with either thiopentone or ketamine to allow intubation

i. Midazolam, pethidine and thiopentone

The median initial dose of thiopentone was 1000 mg (3.4 mg/kg, range = 2.23 - 5.93 mg/kg, n = 10). One animal required an additional dose of 500 mg thiopentone to allow intubation. The doses of pethidine and thiopentone were not significantly different between saline and naloxone groups.

Thiopentone usually produced level 4 - 5 chemical restraint for approximately 5 min with recovery to pre-anaesthetic levels of sedation about 15 min later (shown as the duration of anaesthetic effect in Table 9.1). The maximum level of chemical restraint was not closely related to dose of thiopentone administered ($r_S = 0.25$, $P > 0.4$, n =10), but faster injection appeared to give a greater effect.

With the exception of 3 animals which became apnoeic, heart rate and respiratory rate were usually unaffected by thiopentone administration which allowed access to the larynx for intubation within 5 min of drug administration in all but one case. Intubation did not affect respiratory rate nor did it stimulate breathing in the 3 animals which became apnoeic. Two of these animals commenced breathing when the endotracheal tube was removed, and the third appeared to respond to naloxone. One animal became dangerously deep (level 7) after thiopentone administration. This animal had been excited and aggressive at level 2 and consequently a relatively large dose of thiopentone (7.52 mg/kg) had been rapidly administered. The animal was intubated and immediately given naloxone. Breathing recommenced and the animal recovered to level 2 at 46 min, but relapsed to level 3 at 82 min before eventual recovery.

Before giving saline or naloxone, all monitored responses were similar for 15:1500 midazolam, pethidine, thiopentone and saline and 15:1500 midazolam, pethidine, thiopentone and naloxone groups. After saline or naloxone the heart rate, respiratory rate (Table 9.2), Pv_{O2} (median = 39 mmHg) and Pv_{CO2} (median = 56 mmHg) were similar for the two groups; however, pH (median = 7.30 and 7.22), HCO_3^- (median = 28 and 23 mmol/L), total CO_2 (median = 30 and 25 mmol/L) and base excess (median = -1 and -7 mmol/L) were lower after naloxone (U range = 1.0 - 2.0, $P < 0.05$) (Table 2).

The time to recovery after 15:1500 midazolam, pethidine and thiopentone was similar for both the saline and the naloxone groups (median = 113 min).

ii. Midazolam, pethidine and ketamine

The median initial dose of ketamine was 1000 mg (2.27 mg/kg, range = 0.97 - 3.51 mg/kg, n = 6). One animal required two additional doses of 500 mg ketamine to allow intubation.

Ketamine administration usually induced level 4 chemical restraint for approximately 4 min and animals returned to pre-administration levels 12 min later (Table 9.1).

The median respiratory rate was significantly reduced after ketamine, from 7 breaths/ minute to 4 breaths/ minute ($U = 24.5$, $P < 0.05$). Apnoea of 1 to 2 min was seen on 2 occasions after 1000 mg ketamine administration but ceased when the animals' muzzles were tapped. Two other animals appeared to have increased respiratory effort after ketamine.

All animals were intubated within 5 min of drug administration despite one animal only reaching level 3 of chemical restraint. Apnoea for the duration of intubation was seen in 2 animals which received 500 mg of ketamine (0.97 and 1.58 mg/kg), and there were 2 episodes of transient 1 - 2 minute apnoea immediately after intubation in animals to which 1000 mg was administered.

The animal which received naloxone was at level 3 and moved off at level 2 with a dissociated look approximately 4 min afterwards (Table 9.1). Time to recovery was 90 min which was less than that of the animals which did not receive naloxone (median = 120 min; Tables 9.1 and 9.2).

iii. Comparison of midazolam, pethidine and thiopentone with midazolam, pethidine and ketamine

Doses of midazolam and pethidine were similar for all 15:1500 midazolam, pethidine, thiopentone and saline and 15:1500 midazolam, pethidine, ketamine and saline animals, and heart rate, respiratory rate and pH and blood gas values were also similar for the time period prior to thiopentone or ketamine administration.

After thiopentone or ketamine the magnitude and time course of response to the drugs was similar: level 4 chemical restraint (range = 3-7) was induced within 5 min and lasted approximately 10 min (Tables 9.1 and 9.2). The median heart rate (62 bpm) and respiratory rate (4 breaths/ min) and blood gas values were also similar (Table 9.2) and there was no difference in the incidence of apnoea or difficulty breathing (3/10 after thiopentone and 4/6 after ketamine) (Fischer's Exact Test, $P > 0.18$).

For both groups laryngeal relaxation was good and most animals were easily intubated. The proportion of animals which became apnoeic or had difficulty breathing after intubation was also similar for animals which received ketamine (4/6) compared with those which received thiopentone (3/10) (Fisher's Exact Test, $P > 0.18$) although the apnoea was more easily treated after ketamine.

C. Midazolam and pethidine combined with ketamine for prolonged immobilization

Level 4 - 5 chemical restraint was maintained for about 1 h by administering 500 mg increments of ketamine at approximately 5 - 10 minute intervals, depending upon the response. The median number of 500 mg doses of ketamine required was 4 (range = 3 - 9), and the median time between doses was 8 min (range 3 - 24 min). The median incremental dose of ketamine administered was 1.48 mg/kg (range = 1.47 - 1.52 mg/kg, n = 3), or 7.72 mg/kg/h (range = 4.42 - 10.05 mg/kg/h).

On 3 occasions after ketamine administration respiratory rate slowed or became more shallow, including one 3 minute period of apnoea which resolved after the animal's muzzle was touched. No evidence of hyperthermia was seen in any of the animals in Parts A, B or C.

Discussion

A. Midazolam combined with pethidine for sedation

The combination of midazolam and pethidine at the higher dosage (15:1500 midazolam and pethidine) was very useful for sedation and allowing intravenous access. Although others (Cornell and Antrim 1987, Joseph and Cornell 1988) have gained intravenous access with lower doses of pethidine, their animals were physically restrained.

The chief advantages of 15:1500 midazolam and pethidine were its rapid antagonism, ease of reversal of apnoea and the lack of side effects commonly seen with cyclohexamine drug combinations. Some animals were sufficiently tractable to be rolled onto a stretcher and weighed, which would not have been possible at this level of chemical restraint with cyclohexamine drug combinations.

There were some problems with 15:1500 midazolam and pethidine. Although intravenous access was achieved in all animals, it was difficult in some which were at level 2, and this sometimes necessitated further drug administration. (In other studies, unpublished, we have administered incremental intramuscular doses of 1000 - 1500 mg [or approximately 2 - 4 mg/kg] pethidine safely, and maintained sedation for up to 4 h). The response was slow, as it took up to 20 min to reach level 3 chemical restraint, longer than for most commonly used cyclohexamine-based combinations. Respiration was shallow, which could make the assessment of ventilation difficult and large dosage volumes were required (usually 30 mL, range = 20 - 50 mL).

Naloxone rapidly antagonised 15:750 midazolam and pethidine but did not antagonise 15:1500 midazolam, pethidine and thiopentone (Table 9.1) indicating that the 20:1 ratio may be more appropriate than the 40:1 ratio. However the formulation of naloxone in vials of 0.4 mg (or 2 mg) required many vials to be opened for a 40 mg dose. This was difficult and time consuming and could prove fatally slow under emergency conditions, and a more potent narcotic antagonist would be

preferable. Other results suggest that naltrexone may be useful in these animals but, since not all animals responded, larger doses may need to be used, and dose-response studies are indicated. No more naltrexone was available for this study due to supply difficulties at Macquarie Island.

Given the evidence of relapse after narcotic antagonism in this study it may be preferable to give doses of antagonists both intramuscularly and intravenously and, because of the rapidity with which some animals recovered, to give the intramuscular dose first.

Pethidine was chosen instead of a more potent narcotic because of the decreased chance of self inflicted injury (Anonymous 1976) or drug overdose. For larger animals, especially bulls, or if administering the drugs by projectile syringes, the volume of pethidine required would be prohibitive and a more potent narcotic, or different formulation, required. Several potent narcotics have been administered to pinnipeds; however, most have been associated with complications (such as apnoea, convulsions, and vomiting), unpredictable results and narrow margins of safety (Gales 1989). The safety and usefulness of the potent narcotics could be improved by administering appropriate doses of drug, combining them with reversible sedative drugs, and using them to sedate animals, rather than as the sole source of drug for deeper levels of restraint. If the level of restraint needs to be increased this could be accomplished by intravenous administration of a barbiturate or ketamine, or application of a nose cone and gas, with or without narcotic antagonism.

B. Midazolam and pethidine combined with thiopentone or ketamine to allow intubation

The doses of thiopentone and ketamine induced similar levels of chemical restraint, sufficient for intubation and there appeared to be little difference in the response of animals to each of the drugs. Apnoea or difficulty breathing apeared to be more readily treated after ketamine (by tapping the face) which indicated that ketamine may be preferable to thiopentone for induction prior to intubation.

The dangerously deep level of chemical restraint seen in one animal was evidently because of the large dose of thiopentone and its rapid administration. We are certain that this animal would have died had not naloxone been immediately given, or if the ability to intubate and ventilate the animal had not been available. Fortunately, the 40 mg of naloxone caused arousal, presumably because it reached its sites of action before the circulatory changes associated with apnoea (similar to those seen during diving) prevented its effect (Backhouse 1964). In situations where animals are insufficiently sedated and rapid induction is required it may therefore be preferable to administer a number of relatively low doses of thiopentone (perhaps 1.5 mg/kg boluses) by rapid intravenous injection rather than administering a single large dose rapidly. The other option of slowly infusing the drug to effect also seems unsatisfactory since if intravenous doses are given too slowly effective concentrations of drug may not be reached in the brain.

Given the tractable nature of the animals sedated with midazolam and pethidine, it may be possible to use a mask and gaseous anaesthesia to minimise problems of apnoea and/ or deep anaesthesia during induction. Alternatively, an antagonist could be administered immediately after intubation (following thiopentone or ketamine) to minimise the combined depressant effects of the drugs; the balance of anaesthesia being achieved using gas. If this technique is used the possibility of relapse would need to be considered.

The extent of apnoea and central depression associated with intravenous drug administration in this study was less than expected from literature reports of sudden death after inadvertent intravascular administration of anaesthetics (Gales and Burton 1987a), the depressant effects of narcotics and barbiturates reported in other seals (Gales 1989) and our own previous experience of apnoea after small incremental doses of cyclohexamines. Possible reasons for this include the use in this study of low doses, intubation, antagonists and premedication with midazolam and pethidine. Animals sedated with midazolam and pethidine also appeared very relaxed compared with those sedated with cyclohexamine-based drug combinations. Backhouse (1964) reported that fright may play a role in initiation of apnoea during restraint, and the intravenous administration of appropriate dosages of drugs to relaxed, apparently unstressed animals may thus be important in preventing apnoea.

Intubation under midazolam and pethidine was easier than we have found when using cyclohexamine-based drug combinations, illustrated by the successful intubation of one animal which was at level 3 chemical restraint, a procedure which would not have been possible using the other drugs. This may be due to the analgesic or spasmolytic effects of pethidine (Hall 1959).

The results of this study indicate that use of midazolam and pethidine followed by thiopentone or ketamine was a useful and controllable technique for intubation and could be used prior to gaseous anaesthesia. This was primarily because of the ability to antagonise pethidine: however, there are problems associated with antagonist use in seals. Drugs administered in the extradural intravertebral vein of apnoeic animals may be ineffective (Backhouse 1964, Baker and Gatesman 1985), and intubation and ventilation may be needed.

The relatively long period required to induce adequate sedation (up to 20 min) compared with cyclohexamine drug combinations (10 min) indicates that this technique may be less desirable when large numbers of animals are to be immobilized or conditions are poor.

C. Midazolam and pethidine combined with ketamine for prolonged immobilization

Ketamine was chosen rather than thiopentone for maintaining immobilization because it appeared that apnoea was more easily treated (Part B).

Administration of ketamine was very useful for maintenance of chemical restraint for minor surgical procedures. The use of local anaesthetic and pethidine probably decreased the absolute requirement for ketamine. Care was required with incremental ketamine administration which sometimes caused difficult breathing. It is likely that the prolonged recovery associated with this drug combination could be largely eliminated by routine use of a narcotic antagonist, but this was not tested. Although gaseous anaesthesia may be preferable, ketamine would be useful in situations requiring prolonged chemical restraint with good analgesia where gaseous anaesthesia is impractical or unavailable.

Administration of intravenous boluses of ketamine allowed rapid and accurate adjustment of the level of chemical restraint after 15:1500 midazolam and pethidine. Gales and Burton (1987a) preferred intramuscular administration of further doses of ketamine in southern elephant seals immobilised with ketamine plus diazepam or xylazine because intravenous administration was considered to be often unpredictable and short acting. The potential for overdose and the respiratory depression seen in this study are disadvantages of intravenous ketamine administration. However, the intravenous route is preferable to induce or maintain chemical restraint > level 5 since it can induce a rapid, short term increase in level of chemical restraint.

Use of midazolam and pethidine for sedation and to allow intravenous access for anaesthetic administration, when combined with an accurate system of monitoring, use of narcotic antagonists and the ability to intubate and ventilate animals provided a safe, reversible, versatile technique for chemically restraining mature female southern elephant seals. It is the safest technique we have used for allowing intravenous access in these animals. These techniques have potential for use in other species of seal, but the interspecific variability in response to pethidine, which has been described in other seals (Joseph and Cornell 1988), would need to be considered.

Chapter 10: Medetomidine and ketamine use

Introduction

Medetomidine is a specific α_2 adrenergic receptor agonist having properties which are similar in many respects to xylazine (Savola 1989). However it is more potent (Scheinin and MacDonald 1989), the α_2 / α_1 selectivity ratio is greater (Virtanen 1989), and its anaesthetic dose reducing property far exceeds that seen with other α_2 agonists (Doze *et al.* 1989). (Alpha$_2$ adrenergic agonists markedly reduce ketamine requirements by their central nervous system effects and by increasing ketamine bioavailability. Mechanisms for this effect have been reviewed by Klein and Klide (1989) and may include reduced liver blood flow, direct depression of metabolic function, and decreased urinary excretion). The recommended doses for medetomidine in domestic animals range widely (0.010 - 0.150 mg/kg). At medetomidine doses higher than the recommended ones, the degree of sedation does not increase, only the duration of effect, and vomiting and hypothermia have been associated with its use (Vainio 1989). Elimination rate is controlled mainly by liver blood flow (Salonen 1989).

Ketamine has been combined with medetomidine (ketamine and medetomidine) to immobilize a variety of zoo and domestic animals. Medetomidine potentiates the anaesthetic effects of ketamine and balances its weak muscle relaxing and analgesic effects (Verstegen *et al.* 1989). The cardiovascular stimulating properties of ketamine largely compensate for the bradycardia induced by medetomidine (Verstegen *et al.* 1989).

Atipamezole is one of the most selective and potent α_2 adrenergic antagonists known (Virtanen 1989), and has been used to antagonise ketamine and medetomidine in a variety of animals.

Medetomidine and atipamezole have not been administered to pinnipeds. Ketamine and medetomidine offers advantages over ketamine and xylazine and other cyclohexamine based drug combinations, including smaller doses of ketamine, reversibility, and fewer side effects. Medetomidine could also be useful for sedation of large seals, its chief advantages being its potency, small dose volume and ability to be antagonised without the risk to the operator of death (Anonymous 1976) or the legal restrictions associated with the use of potent narcotic drugs. A successful trial with elephant seals would suggest it may also be useful for leopard seals (*Hydrurga leptonyx*), a species in which xylazine use has resulted in fatalities (Mitchell and Burton 1991).

A pilot study was conducted to assess its usefulness.

Materials and methods

Twelve healthy, quiescent, pre-moulting females of median 2.31 m snout-tail length (range = 2.08 - 2.70 m) and median mass 355 kg (range = 270 - 445 kg) were used. There were three parts to the study.

Sedation with medetomidine

To assess the effect of medetomidine on the animals and determine a likely starting dose for combination with ketamine, two animals were sedated with a single intramuscular dose of medetomidine; one received 5 mg the other 10 mg. The dose of medetomidine was based on the lowest dose recommended for dogs (0.010 mg/kg; Vainio 1989).

Medetomidine and ketamine

Ten animals, in two groups of 5 animals, received ketamine and medetomidine. Twenty minutes after the administration of ketamine and medetomidine each group received a similar volume of either atipamezole or saline intravenously into the extradural intravertebral vein. The intended dose of ketamine (1.5 mg/kg or 675 mg; Table 10.1), was based upon that used to restrain southern elephant seals with ketamine and xylazine (Chapter 8), but was decreased because of the anaesthetic reducing properties of medetomidine (Doze *et al.* 1989). One animal received 10 mg medetomidine, the others 5 mg, and the dose of atipamezole was approximately twice (w/w) the medetomidine dose as used in other animals (Jalanka 1988 and 1989).

Atipamezole was administered at 20 minutes because it was considered that most routine procedures performed in these animals could be completed in this time.

Each animal in Parts 1 and 2 received 1.3 mg atropine combined with the anaesthetic drugs.

Comparison of ketamine and medetomidine with ketamine and xylazine and other cyclohexamine based drug combinations

Where sample sizes were large enough, data describing the response to ketamine and medetomidine in the present study were compared with data describing the response to ketamine and xylazine in a previous study (see Chapter 8).

Anaesthetic drugs were administered intramuscularly after the technique of Ryding (1982) and a standard monitoring sheet completed for each animal (Chapter 3 and 4).

Table 10.1. Dose of drugs administered to female southern elephant seals, response variables (median and range), and incidence of prolonged chemical restraint. Data for animals restrained with ketamine and xylazine are included for comparison. The median dose of atipamezole was 0.04 mg/kg (range = 0.02 - 0.05 mg/kg). None of the values recorded for ketamine and medetomidine and ketamine, medetomidine and atipamezol were significantly different (U = result of Mann-Whitney U test.)

Treatment	Number of animals	Drug dose (mg/kg)				Maximum level of chemical restraint			Time to (min)		Level chemical restraint at first movement forward	Number of episodes of	
		Anaesthetic (ketamine)		Sedative (medetomidine or xylazine)		Maximum level	Time to (min)	Duration of (min)	Recovery	First movement forward		Prolonged restraint	Suspected hyperthermia
		Intended	Actual	Intended	Actual								
Medetomidine	2			0.01*	0.0196*	1.5*	10*	24*	45*	40*	1.5*	0	1
Ketamine and medetomidine	5	1.50	1.90 (1.35-2.23)	0.01	0.017 (0.012-0.027)	3 (1-3)	12 (10-25)	22 (5-43)	87.5 (15-120)	41 (40-120)	1 (1-2)	3	2
Ketamine and medetomidine and atipamezol	5					3 (2-6)	11 (5-18)	13 (7-18)	31 (23-180)	30 (25-80)	2 (1-3)	2	4
Ketamine and xylazine†	15	3.00	2.70 (2.06-3.14)	0.50	0.48 (0.22-0.54)	4 (2-6)	9 (3-6)	15 (5-55)	91.5 (40-191)	59 (27-81)	2 (1-3)	12	8
			U = 3.0‡ P < 0.01			U = 6.5‡ P < 0.01							

*Average values. For individual values see text.

†Data from Chapter 8.

‡Comparison of ketamine and medetomidine and ketamine and xylazine.

Results

Sedation with medetomidine

The animal which received 10 mg (0.027 mg/kg) medetomidine became more heavily sedated (maximum level of chemical restraint = 2 and 1), had a lower median heart rate for the first 60 minutes (36 and 60 beats/ min; bpm), and took three times as long to recover fully (level 0 restraint; 180 and 60 min) than the animal which received 5 mg (0.013 mg/kg) medetomidine. Respiratory rate for the first 60 minutes were similar (median = 4 and 3 breaths/ min; ranges = 2 - 4, and 3 - 5 breaths/ minute respectively) for the two seals.

Muscle relaxation was profound in both animals, and they appeared less responsive to nociceptive stimuli than animals anaesthetised with other cyclohexamine based drug combinations at similar levels of chemical restraint. However, neither was sedated sufficiently to allow intravenous access. The animal which received 10 mg responded very aggressively to monitoring and showed evidence of hyperthermia, while the other animal which had received 5 mg, vomited.

Medetomidine and ketamine

The doses of ketamine (median = 1.90 mg/kg, range = 1.35 - 2.23 mg/kg) and medetomidine (median = 0.017 mg/kg, range = 0.012 - 0.027 mg/kg) administered to each of the ketamine and medetomidine groups were similar. The dose of atipamezole was 0.04 mg/kg (range = 0.02 - 0.05 mg/kg). The response to ketamine and medetomidine was inconsistent and unpredictable.

Administration of 10 mg medetomidine with ketamine to one animal caused light anaesthesia (level 6). There was a transient 10 minute period of apnoea, and the median heart rate for the first 60 minutes was 30 bpm (range = 24 - 48 bpm). The 5 mg dose of medetomidine combined with ketamine (administered to all other animals) was usually associated with higher heart rates (median = 48 bpm, range = 30 - 72 bpm), and lower maximum levels of chemical restraint (median = 3, range = 1 - 6).

Administration of atipamezole had little effect upon the level and time course of chemical restraint (Table 10.1). The median levels of chemical restraint, heart rate and respiratory rate for the 20 minute period after atipamezole were similar to those values for the same period after saline.

Evidence of hyperthermia was seen in 2 ketamine and medetomidine, and 4 ketamine and medetomidine/ atipamezole animals. Aggressive reactions, which included growling, biting and lunging at the operators, were seen in 4 ketamine and medetomidine and 3 ketamine and medetomidine/ atipamezole animals and prolonged chemical restraint (> 60 minutes until recovery;

level 1 restraint) was seen in 2 ketamine and medetomidine and 2 ketamine and medetomidine/ atipamezole animals.

Comparison of ketamine and medetomidine with ketamine and xylazine and other cyclohexamine based drug combinations

Response, monitoring and blood gas values for animals restrained with ketamine and medetomidine, ketamine and xylazine and grouped values for five commonly used cyclohexamine based drug combinations (100:1 and 50:1 ketamine: diazepam, ketamine and midazolam; ketamine and xylazine, and tiletamine and zolazepam; from Chapter 8) are presented in Tables 10.1, 10.2 and 10.3.

The median dose of ketamine used with medetomidine in this study (1.90 mg/kg) was lower than the median dose of ketamine used with xylazine in the study in Chapter 8 (2.70 mg/kg), and ketamine and medetomidine animals were less sedated (Table 10.1). However the responses to ketamine and medetomidine and to ketamine and xylazine were similar in time course of response, level of chemical restraint at first movement forward, and number of episodes of prolonged restraint and suspected hyperthermia (Table 10.1). Ketamine and medetomidine animals appeared more depressed than ketamine and xylazine animals (heart rate and respiratory rate were lower) however ketamine and medetomidine animals appeared to react more aggressively to being touched and median values for head response, muscle tone, and righting response were greater (Table 10.2). Blood gas values recorded at levels 3 and 4 restraint appeared similar for ketamine and medetomidine and ketamine and xylazine animals except that values recorded for Pv_{O2} were higher for ketamine and medetomidine animals (Table 10.3).

Discussion

Sedation with medetomidine

Because of the bradycardia reported in animals using xylazine (a less potent α_2 adrenergic agonist) combined with ketamine (Chapter 8) we were concerned about the effect of the medetomidine on circulation. For this reason low doses of medetomidine were chosen. The dose of medetomidine was too low to allow intravenous access and would have to be increased if medetomidine was being used as the sole agent for chemical restraint. However, given the relatively low heart rate associated with administration of 10 mg medetomidine (36 bpm), any increase in dose should be done with caution. Premedication of animals with a parasympatholytic drug prior to medetomidine administration, rather than the simultaneous administration commonly used, may lessen this effect. Räihä *et al.* (1989) found that atropine (0.04 mg/kg) counteracted medetomidine induced bradycardia in dogs and recommended its use prior to medetomidine administration.

Table 10.2. Descriptive statistics (median and range) for each response used in monitoring at level 3 or 4 of chemical restraint for seals restrained with ketamine and medetomidine. Data for animals restraint with ketamine and xylazine and grouped data for animals restrained with 100:1 ketamine: diazepam, 50:1 ketamine: diazepam, ketamine and midazolam and tiletamine and zolazepam are included for comparison. Neither of the 2 animals sedated with medetomidine reached level 3 or 4 chemical restraint.

Drug group	Number of animals	Response								
		Heart rate (beats/ min)	Respiratory rate (breaths/ min)	Temperature (°C)	Head response	Palpebral response	Withdrawal response	Caudal flipper response	Muscle tone	Righting response
Ketamine and medetomidine	3*	40 (31-48)	2 (1.5-5)	38.5 (38.0-39.0)	3 (2-3)	3 (3-3)	0 (0-1)	1 (0-1)	2 (0-2)	2 (2-2)
Ketamine and xylazine†	15	50 (37-61)	4.5 (3-6)	37.1 (36.8-38.0)	4 (3-4)	2 (2-3)	1 (0-3)	1 (0-3)	1 (0-3)	1 0-3)
100:1 ketamine: diazepam, 50:1 ketamine: diazepam, ketamine and midazolam, ketamine and xylazine and tiletamine and zolazepam†	75	59 (37-82)	5 (1-10)	36.9 (35.0-38.0)	3 (2-4)	3 (1-3)	1.5 (0-3)	1 (0-3)	2 (0-3)	2 (0-3)

*Only 3 animals which received ketamine and medetomidine reached levels 3 or 4.
†Data from Chapter 8.

Table 10.3. Descriptive statistics (median and range) for each blood gas variable at level 3 or 4 of chemical restraint for seals restrained with ketamine and medetomidine. Values are presented for seals restrained with ketamine and xylazine and grouped values for animals restrained with 100:1 ketamine: diazepam, 50:1 ketamine: diazepam, ketamine and midazolam, ketamine and xylazine, and tiletamine and zolazepam for comparison.

Drug group	Number of animals	Blood gas variable					
		pH	PvO2 (mmHg)	PvCO2 (mmHg)	HCO3- (mmol/L)	Total CO2 (mmol/L)	Base excess (mmol/L)
Ketamine and medetomidine	3*	7.30 (7.30-7.31)	50 (46-54)	47 (41-51)	24 (21-26)	26 (22-28)	-2 (-5-0)
Ketamine and xylazine†	15	7.31 (7.24-7.36)	40 (34-49)	50 (38-57)	26 (20-30)	28 (22-32)	-2 (-5-2)
100:1 ketamine: diazepam, 50:1 ketamine: diazepam, ketamine and midazolam, ketamine and xylazine and tiletamine and zolazepam†	75	7.32 (7.22-7.42)	43 (20-56)	50 (30-68)	26 (16-35)	28 (17-37)	-1 (-8-7)

*Only 3 animals which received ketamine and medetomidine reached levels 3 or 4.
†Data from Chapter 8.

Atropine has been administered at a dose of approximately 0.005 mg/kg to southern elephant seals (Gales and Burton 1987 a, Woods *et al.* 1989, Mitchell and Burton 1991) which is a low dose relative to the range of doses administered to other animals as premedicants (0.045 - 0.200 mg/kg; Booth 1982 f) or seals (0.02 - 0.04 mg/kg; Sweeney 1974, Hammond and Elsner 1977). Atropine is known to prevent the bradycardia associated with the dive response in northern elephant seals, *Mirounga angustirostris* (Van Citters *et al.* 1965), and a dose of 0.02 - 0.04 mg/kg has been recommended (Sweeney 1974, Hammond and Elsner 1977). This higher dose may counteract the medetomidine induced bradycardia in southern elephant seals. However, atropine use for blocking xylazine-induced bradycardia in other animals has been considered controversial (Klein and Klide 1989). Raising the heart rate in the presence of increased cardiac afterload increases myocardial oxygen demand while cardiac output and oxygen supply are reduced after xylazine administration. Studies determining the efficacy of atropine in southern elephant seal anaesthesia are required.

The only advantage medetomidine appeared to have over xylazine when used alone for restraint was in its greater potency and a formulation which would allow its delivery using projectile syringes. The vomiting, hyperthermia, aggression and low heart rate associated with medetomidine administration in this study have also been seen when xylazine was used to sedate southern elephant seals (Vergani 1985). Given these side effects it is unlikely that medetomidine when used alone, will prove to be a useful drug for immobilization of larger southern elephant seals or leopard seals.

Medetomidine and ketamine

Although ketamine and medetomidine produced a median level 3 of chemical restraint, the response was unpredictable; animals receiving similar doses reached level 1 and level 6. Because the dose of 10 mg of medetomidine combined with 675 mg ketamine caused excessive restraint and decreased heart rate in the first animal to which it was administered, subsequent animals received 5 mg medetomidine combined with ketamine. However, as animals were usually insufficiently restrained (< level 3) rather than excessively deep (> level 5) the dose of ketamine or medetomidine in this combination may need to be increased. Because the animal which received 10 mg of medetomidine had a low heart rate, it might be preferable to either use this dose of medetomidine and decrease the dose of ketamine, or to use a lower dose of medetomidine and increase the dose of ketamine, rather than increase the medetomidine dose. (One study found that increasing the dose of ketamine cancelled the bradycardia related to medetomidine administration (Verstegen *et al.* 1989) so the second option may be preferable).

The failure of atipamezole to antagonise ketamine and medetomidine was unexpected, and was possibly because the dose of atipamezole was too low. Given the occasional relapse to sedation seen after intravenous administration of atipamezole in other animals (Jalanka 1989) it may be more useful to administer the drug divided intravenously and subcutaneously. Atipamezole was given intravenously in the present study to assess its duration of effect and look for evidence of relapse. As

atipamezole antagonizes some of the effects of medetomidine in other animals (Savola 1989, MacDonald *et al.* 1989) it is likely that the adverse side effects seen during this study could be reduced if a suitable dose of antagonist was administered.

Comparison of ketamine and medetomidine with ketamine and xylazine and other cyclohexamine based drug combinations

The small sample sizes, difference in maximum levels of chemical restraint, and use of non-standardised drug dosages in the ketamine and medetomidine and ketamine and xylazine studies mean that few direct comparisons or conclusions can be made between the combinations. A dose-response study had been planned to compare these two combinations but was cancelled because of the apparent lack of benefit of ketamine and medetomidine compared with ketamine and xylazine found during this pilot study. However, the response to ketamine and medetomidine appeared to be similar in many ways to the response to ketamine and xylazine; sedation and muscle relaxation were profound; heart rate was relatively low compared to other cyclohexamine based combinations; aggressive responses to monitoring and unwanted side effects such as hyperthermia, prolonged restraint and vomiting (which has been associated with ketamine and xylazine use in other studies; Mitchell and Burton 1991) were all seen. Consequently ketamine and medetomidine appeared to offer few advantages over ketamine and xylazine or other cyclohexamine based drug combinations for routine chemical restraint. However the results also suggested that medetomidine may decrease the dose of ketamine required, as it does in other species (Doze *et al.* 1989), which could be an advantage if a suitable dose of drug and antagonist was administered.

The role of α_2 adrenergic agonists in chemical restraint of pinnipeds is debatable. There are many reports of adverse side effects associated with their use including hyperthermia, vomiting, bradycardia, apnoea, death and prolonged restraint (Vergani 1985, Woods *et al.* 1989, Mitchell and Burton 1991, Chapter 8). Woods *et al.* (1989) suggested that excessive doses of xylazine were implicated in initiation of diving responses in anaesthetised seals. Despite these problems, ketamine and xylazine has been used successfully on many occasions (Woods *et al.* 1989) and the ability to antagonise the adrenergic α_2 agonists is a distinct advantage. To minimise these side effects it may be expedient to combine them with other drugs, allowing a reduction in their dosage, rather than relying on them as the sole source of drug for immobilization. Prior premedication with a parasympatholytic may be useful. It is also possible that their usefulness and safety could be improved by combining them with other easily antagonised drugs (such as narcotics) which could allow rapid antagonism of effects and lower the requirements of each component. However, possible additive depressant effects of these drugs, and the inability to antagonise drugs when circulation has shut down (Chapter 11 and 12) would need to be considered and their use approached with caution.

Because the sedative effects of α_2 adrenergic agonists vary considerably between species (Stenberg 1989), caution would need to be exercised in attempting to extrapolate doses of medetomidine used in this study to those which may be useful in other pinniped species.

Chapter 11: Use of the respiratory stimulant doxapram

Introduction

Apnoea is commonly associated with chemical restraint of southern elephant seals (Mitchell and Burton 1991) and other pinnipeds (Gales 1989), but there are few specific recommendations regarding its treatment. The respiratory stimulant doxapram (Parry *et al.* 1981, Cornell and Antrim 1987, Baker *et al.* 1988, Bester 1988) and intubation and positive pressure ventilation (Baker *et al.* 1990) have been used. Doxapram's stimulatory effect in other animals is related to direct action upon chemoreceptors of the carotid and aortic regions. There may also be some stimulation of the medullary respiratory centre (Booth 1982b). In the studies above in which doxapram was used, few or no data were presented and intubation and positive pressure ventilation cannot be used effectively in all circumstances. For example, logistical problems associated with restraining animals in isolated, inaccessible locations may preclude the use of bulky or delicate resuscitation equipment. Even if this equipment is available there are problems associated with ventilation of large animals which have large tidal and respiratory minute volumes. To deliver the required volume of gas within the desired time span increased and/ or prolonged elevated mean airway pressure may be required and this can cause significant depression of cardiac function by decreasing thoracic venous inflow, stroke volume, cardiac output and aortic blood pressure (Steffey 1983 a). In phocid seals ventilation equipment also has to inflate the lungs against the weight of the compressible thorax (Hammond and Elsner 1977); an important consideration in southern elephant seals which can weigh up to 3700 kg (Ling and Bryden 1992). For these reasons effective ventilation of large seals ($\geq$ 1000 kg) may be difficult to achieve and doxapram may offer an alternative.

Two aims of this study were thus: (1) to determine whether doxapram was a useful treatment of apnoea and (2) to determine the optimum dose and route of administration. As the study proceeded results indicated that endotracheal intubation of some study animals could be affecting breathing (see below). A third aim was therefore (3) to examine the effect of intubation on respiratory rate in southern elephant seals.

Materials and methods

There were three parts to the study. Part A refined the dose of doxapram, Part B its route of administration and Part C examined the effect of intubation on breathing.

A. Dose of doxapram in eupnoea

Twenty five healthy, quiescent, pre-moult females of median 2.56 m snout-tail length (range = 2.43 - 2.81 m) and median mass 354 kg (range = 301 - 613 kg) were immobilized (level 4 of chemical restraint; Table 11.1 footnote) with a single intramuscular dose of ketamine and xylazine. The median dose of ketamine was 3.0 mg/kg (range = 1.6 - 3.5 mg/kg) and of xylazine was 0.5 mg/kg (range = 0.3 - 0.7 mg/kg).

Twenty min after ketamine and xylazine administration each group of seals was given a single fixed dose of doxapram or saline of 100mL volume, warmed to 37°C, intravenously into the extradural intravertebral vein. The seals were randomly assigned to five groups of five which received either 250 mg, 500 mg, 1000 mg, or 1600 mg doxapram or saline (control) (Table 11.1).

Respiratory rate was recorded each minute after doxapram or saline until it returned to 4 to 5 breaths/min. From these data the duration of increase in respiratory rate, the maximum respiratory rate and its time were determined.

B. Route of administration in apnoea

The optimal dose of doxapram found above (1000 mg) was then assessed as a respiratory stimulant in 20 mature female pre-moult southern elephant seals which had become apnoeic for 10 min or longer whilst chemically restrained (median level chemical restraint = 4, range = 3 - 6) with ketamine and xylazine. The median dose of ketamine was 3.3 mg/kg (range = 2.5 - 3.4 mg/kg) and the median dose of xylazine was 0.5 mg/kg (range = 0.5 - 0.6 mg/kg). Ten min after the onset of apnoea, animals were treated with either doxapram or saline (50 mL, 37°C), administered by one of 3 different routes (Table 11.2):

1. Intralingually: Injections were made intramuscularly into the body of the tongue, by inserting an 18 G, 38 mm needle through the lateral surface. Ten mL of fluid was the maximum administered in any one spot before the needle was redirected or removed and relocated. No attempt was made to test for intravascular administration.

2. Intravenously: Injections were made into the extradural intravertebral vein (as above).

3. Endotracheally: Each animal was intubated with a 24 mm outside diameter endotracheal tube (not inflated) and a sterile duodenal tube, passed down the lumen of the endotracheal tube and advanced 5 - 10 cm past the distal end of the endotracheal tube. A syringe containing doxapram or saline was attached to the proximal end of the duodenal tube and the contents administered rapidly. The syringe

Table 11.1. Results of Part A. Dose of doxapram (intended and actual), respiratory rate before experimental treatment, maximum respiratory rate after treatment, duration of increased respiratory rate after treatment (time taken for respiratory rate to return to pre-treatment level) and level of chemical restraint before and after treatment (median, range) for 5 groups of southern elephant seals. Values between groups were compared using Kruskall-Wallis H test; values not significantly different have been grouped.

Group	Dose of doxapram		Respiratory rate			Level chemical restraint		n
	Intended* (mg/kg)	Actual (mg/kg)	Before treatment (breaths/ minute)	Maximum after treatment (breaths/ minute)	Duration of increase after doxapram(min)‡	Before treatment§	After treatmentII	
Control	0.0	0.00	5 (1-7)	4 (2-6)	-	4 (2-6)	5 (1-7)	5
250 mg doxapram	0.5	0.75 (0.41-0.77)		6 (5-10)	2 (2-5)		2 (2-3)	5
500 mg doxapram	1.0	1.42 (0.82-1.51)		10 (6-14)	2 (0-3)		3 (2-4)	5
1000 mg doxapram	2.0	2.98 (2.61-3.33)		10 (1-16)	3 (0-7)		3 (3-3)	5
1600 mg doxapram	4.0	4.19 (2.63-4.90)		12 (12-16)	4 (4-7)		1 (1-3)	5
		H = 16.92 df = 3† P < 0.001		H = 13.70 df = 4 P < 0.01	H = 15.26 df = 4 P < 0.01		H = 11.59 df = 4 P < 0.05	

*All dose volumes were 100 mL.

†Excluding saline group.

‡Time taken for respiratory rate to return to pre-administration level.

§A single value was generated for each animal by taking the median of values at 10, 15 and 20 minutes.

IIA single value was generated for each animal by taking the median of values at 25, 30, 35 and 40 minutes.

Level of chemical restraint
1 = Light sedation
2 = Moderate sedation
3 = Heavy sedation
4 = Light immobilizatior

Table 11.2. Results of Part B. Effect of doxapram (2 mg/kg) or saline on respiratory rate of three groups of apnoeic southern elephant seals chemically restrained with ketamine and xylazine.

Route of administration	Treatment	Effect on respiratory rate (number of animals)	
		Increased	No increase
Intralingual	Doxapram	1	2
	Saline	1	0
Extradural intravertebral vein	Doxapram	0	5
	Saline	0	3
Endotracheally	Doxapram	4	2
	Saline	0	2

was removed and the duodenal tube flushed with 50 mL of air. The duodenal tube was then removed and the endotracheal tube left in place.

C. Effect of intubation

It appeared that the presence of the endotracheal tube in the airway prevented breathing in Part B. It was theorised that if this were the case then removal of the endotracheal tube immediately after doxapram administration, rather than leaving it in place, might improve the effectiveness of the doxapram. This was investigated together with an assessment of the effect of intubation on breathing.

The trial was carried out in mature female pre-moult southern elephant seals which had been chemically restrained (median level chemical restraint = 4, range = 3 - 6) with ketamine and xylazine. The median dose of ketamine was 3.1 mg/kg (range = 2.4 - 6.4 mg/kg) and the median dose of xylazine was 0.5 mg/kg (range = 0.4 - 0.7 mg/kg).

The animals used had either been breathing normally for 10 min (n = 31) or had been apnoeic for 10 min (n = 19), after which each animal was intubated and doxapram, saline or nothing administered endotracheally. The endotracheal tube was then either removed immediately or left in place for 10 min, giving 2 endotracheal tube groups (Table 11.3). For each group the effect of intubation and/ or treatment with doxapram or saline upon respiration was recorded. For those animals in which the endotracheal tube had been left in place for 10 min, the effect of its removal upon respiration was also recorded and is presented as a third group in Table 11.3.

Endotracheal doxapram/ saline were administered as above.

General

For each episode of chemical restraint blood gases and pH (taken from the extradural intravertebral vein), respiratory rate and level of chemical restraint were measured every 5 min for the first 50 min and, if necessary, every 10 min thereafter. The technique used for pH and blood gas analysis has been presented in Chapter 3.

If at any stage during trials there was concern for an animal's welfare, it was immediately intubated and ventilated with oxygen.

Anaesthetic drugs were administered by a remote injection technique (Ryding 1982) and each animal received 1.3 mg atropine sulphate in the same syringe as the ketamine and xylazine.

Table 11.3. Results of Part C. Effect of intubation on apnoeic and breathing animals (number of times that breathing started or improved after treatment/ number of animals in which the treatment was used). The effect of endotracheal tube removal on animals in the second group is also presented (group 3).

Endotracheal tube group	Treatment					
	Apnoeic			Breathing*		
	Saline	Doxapram	None	Saline	Doxapram	None
1. Immediate removal of endotracheal tube after treatment	1/1	3/7†	-	2/3	1/1	-
2. Endotracheal tube left in for 10 minutes after treatment	0/2	4/6†	1/3	0/1	4/5	0/21
3. Effect of endotracheal tube removal on the animals in group 2	1/2	6/6	0/3	0/1	2/5	20/21

*When intubated breathing stopped or became more difficult in 24 of the 31 breathing animals.
†There was no significant difference in the proportion of animals which commenced breathing after doxapram whether the endotracheal tube was removed or left in place (see Text).

Data were analysed using the Mann-Whitney U test to compare two groups, and the Kruskall-Wallis H test for comparison of three or more groups. Correlations were examined with Spearman's r (r_S), and Fisher's exact tests to compare frequencies. Tests were considered significant when $P < 0.05$.

Results

A. Dose of doxapram in eupnoea

The masses of animals in each doxapram group, the doses of anaesthetic drugs and the median levels of chemical restraint and respiratory rate prior to doxapram administration were not significantly different. The actual doses of doxapram administered to the groups were significantly different (Table 11.1).

Doxapram usually caused a transient increase in respiratory rate and depth which commenced within 30 - 60 s of its administration, was maximal after 2 min and lasted less than 5 min. At high doses doxapram caused arousal (see below).

The duration of increase in respiratory rate rose from 2 to 4 min with the dose of doxapram ($r_S = 0.56$, $n = 20$, $P < 0.05$). The maximum respiratory rate after doxapram was significantly different among groups and increased with dose ($r_S = 0.57$, $n = 20$, $P < 0.01$). By 25 min the respiratory rate of doxapram animals had returned to control values: the median respiratory rate for all groups was 5 breaths/min (range = 2 - 13 breaths/min) and it remained unchanged to 40 min.

The median level of chemical restraint was also lower after doxapram (1 - 3) than for the control group (Table 11.1).

Tidal volume appeared to increase with dose of doxapram though this was not quantified. In some animals the inspiratory phase appeared to be lengthened, and animals would often inspire through both nostrils rather than the more usual one. Excursions of the body wall associated with inspiration were also often greater. Despite the changes in respiration doxapram had little effect on venous blood gas values at 25 min which were not significantly different between the five groups. Median (range) of values were: pH = 7.30 (7.23 - 7.39), P_{VO2} = 45 mmHg (30 - 58 mmHg), P_{VCO2} = 50 mmHg (41 - 70 mmHg), HCO_3^- = 26 mmol/L (20 - 32 mmol/L) (n = 24; one animal had recovered and samples could not be taken).

Shaking and aggression were seen in 3 animals given 1600 mg doxapram but not in any other animals.

B. Route of administration in apnoea

Table 11.2 shows the lack of effect of extradural intravertebral vein doxapram. Intralingual doxapram appeared to stimulate breathing in 1 of 3 animals, but in this case respiration commenced almost immediately the needle was inserted into the tongue and before doxapram could be administered. When administered endotracheally doxapram stimulated breathing in 4 of 6 animals. One animal in which doxapram did not stimulate breathing appeared to be consciously breathholding as its external nares were twitching and it was watching the operators as they moved around it. When the endotracheal tube was removed it immediately commenced breathing.

C. Effect of intubation

The number of times that breathing was stimulated in apnoeic animals after treatment with doxapram was similar whether the endotracheal tube was left in place (4 of 6) or immediately removed (3 of 7; Fisher's exact test; P = 0.5) (see † Table 11.3).

The process of intubation caused respiratory difficulties or apnoea in 24 of the 31 breathing animals and stimulated breathing in 1 of the 19 apnoeic animals (Table 11.3). When the endotracheal tube was removed breathing started or improved in 20 of 21 of the breathing animals which did not receive any other treatment and had been intubated for 10 min (Table 11.3).

Discussion

A. Dose of doxapram in eupnoea

It was not possible to measure tidal and minute volume because placement of an endotracheal tube and flow meter tended to cause apnoea. However the increase in respiratory rate and depth seen after all doses of doxapram show that it stimulates normal (spontaneous) breathing in southern elephant seals chemically restrained with ketamine and xylazine (Table 11.1).

The range of doses used in this study included the therapeutic range recommended for intravenous use (0.4 - 2 mg/kg; Booth 1982 b), and that shown to cause arousal (4 mg/kg; Soma and Kenny 1967) in other animals. Although higher doses of doxapram produced a greater increase in the magnitude and duration of respiratory rate, shaking and aggression were seen. A dose of 2 mg/kg provided a better balance between stimulation of respiration and side effects.

Given the increase in respiratory rate, the lack of significant difference in pH and blood gas values at 25 min was surprising but could be due to the transient nature of the changes in respiratory rate and tidal volume and the fact that venous blood gas and pH values may not accurately reflect arterial values (Haskins 1977).

The reversal of ketamine and xylazine chemical restraint by doxapram is consistent with the observation of Parry *et al.* (1981) that doxapram aided recovery of grey seals from narcosis with etorphine and acepromazine. Our results indicate that doxapram could be used to decrease the level of chemical restraint (at 2 mg/kg) or cause arousal (at 4 mg/kg) in animals restrained with ketamine and xylazine.

B. Route of administration in apnoea

Different routes of doxapram administration were trialled because there was some evidence which suggested that drugs administered into the extradural intravertebral vein of apnoeic seals may be ineffective (Baker and Gatesman 1985). This was thought to be due to low pressures and flows in this vessel associated with adaptations to anoxia during diving.

The 1000 mg dose of doxapram was used in Parts B and C because it stimulated respiration without causing shaking, recovery or aggressive behaviour (Part A). This volume of fluid was not considered excessive to be administered into the lungs since 50 mL of saline is routinely used for transtracheal aspiration of horses (Rose 1983).

It is clear that extradural intravertebral vein administration of doxapram cannot be relied upon to treat prolonged apnoea (Table 11.2). However, it increased respiratory rate and depth when administered into the extradural intravertebral vein of breathing animals (Table 11.1) which implies that the failure to have an effect in apnoeic animals is not due to an inherent insensitivity to doxapram. Three theories could explain this lack of effect. Firstly, as suggested by Baker and Gatesman (1985), due to changes in flow in the extradural intravertebral vein during apnoea the drug may not reach its site of action. Secondly, the doxapram may reach its site of action but either a conscious decision not to breathe, or thirdly, the inability to breath due to reflex laryngeal spasm, might "over-ride" the effect of the drug.

The theory of Baker and Gatesman (1985) appears the most simple and probable and if this is the case then to have an effect in apnoeic animals doxapram (and other drugs) would have to be administered by this route before changes in circulation occur, similar to those seen during diving. This is supported in other studies (unpublished) in which we have administered diazepam via the extradural intravertebral vein to seizuring seals which have been apnoeic for a very short period of time (30 seconds to 2 min); in all cases it has been effective indicating that circulation was sufficient to deliver it at a sufficient concentration to the brain (its site of action in other animals).

However, also in other studies (unpublished) we have administered doxapram via the extradural intravertebral vein to apnoeic seals and induced what appeared to be attempts at breathing (ie body heaving) without inspiration of air. When the arytenoids were adducted breathing commenced. We have also seen evidence in apnoeic seals to which doxapram had been administered which suggested

that it was having an effect by increasing level of arousal and causing twitching of the external nares (interpreted as indicating imminent return to breathing) but breath-holding was maintained.

The results of the present study, coupled with these findings make interpretation of the effectiveness of docapram very difficult. The key lies in determining blood flow during apnoea in anaesthetised animals and in examining the control of breathing. Future studies into anaesthesia of these animals should address these areas as a matter of priority.

The failure of doxapram to stimulate respiration when administered intralingually does however cast doubt on the suggestion of Baker and Gatesman (1985) that this route might rapidly deliver drug into the circulation during apnoea. The fact that breathing was stimulated by saline and by placement of the needle within the body of the tongue prior to doxapram administration in the present study implies that the stimulation of breathing seen in this study was due to pain and not the doxapram.

Endotracheal administration was the most effective route of doxapram administration, and allowed maintenance of an airway and the potential to administer positive pressure ventilation. However, it was not effective in all cases and for this reason intubation and ventilation are the treatments of choice for apnoea. Though equipment is available for ventilation of animals < 1000 kg, we are unaware of any commercially available equipment able to effectively ventilate animals ≥ 1000 kg. However it has been suggested that such equipment could be built (F. M. Bird personal communication 1989) and effective techniques for ventilation of large seals are required.

Other routes were considered to deliver doxapram into the heart-brain circulation including intracardiac injection. However, though drugs have been administered by this route during anaesthetic emergencies in other animals (Lumb and Jones 1984 b), this was considered impractical as it would require long needles and large numbers of people to position the seals.

C. Effect of intubation

The small sample sizes, differences in drug dose rates, levels of chemical restraint, total durations of apnoea and treatments at different times mean interpretation of these results is difficult. However, it appeared that the presence of an endotracheal tube tended to compromise breathing in animals which were at low levels of chemical restraint (< 6; light anaesthesia). This is also commonly seen in other animals in which anaesthesia is light (Lumb and Jones 1984 c). This response could also be exacerbated in an animal which must protect its airway whilst diving, and feeding under water. It was therefore of interest that immediate removal of the endotracheal tube after doxapram did not appear to stimulate breathing in more cases than when the endotracheal tube was left in place. This implies that it is not critical whether the endotracheal tube is left in place or removed when doxapram is administered endotracheally. However, leaving the endotracheal tube in place maintains the airway and allows positive pressure ventilation (see below for recommendations). It could also be interpreted as

suggesting that doxapram will "over-ride" any inhibitory effects which the presence of the endotracheal tube may have. Though the data suggest this we would caution against this interpretation; given our current knowledge doxapram cannot be relied upon for treatment of apnoea in southern elephant seals regardless of route of administration.

In summary, if apnoea occurs in animals which are at a low level of chemical restraint (eg < 6) in which there is little evidence of central depression (eg capillary refill and palpebral response are rapid and brisk, mucous membrane colour is good and heart rate relatively normal) intubation can be postponed and other techniques used to stimulate breathing. If animals are at greater levels of chemical restraint (> 5), or there is marked central depression, cyanosis, rapid changes toward deeper levels of chemical restraint, rapid changes in heart rates, or animals cannot be accurately assessed and there is concern for their welfare, then intubation and ventilation are advisable if it is safe to do so.

The endotracheal tube can be removed once the animal commences spontaneous respiration, or when it reaches level 3 - 4 chemical restraint and there is evidence that the endotracheal tube is preventing respiration (ie twitching of external nares or gagging or heaving body movements). Though not always effective, routine administration of doxapram down the endotracheal tube immediately prior to extubation might assist return to spontaneous ventilation.

If positive pressure ventilation cannot be supplied endotracheal administration of doxapram may be helpful; the endotracheal tube being left in place in deep animals (> level 5), or removed when signs of imminent return to breathing occur in light animals (< level 6).

Chapter 12: Antagonism of some cyclohexamine based drug combinations

Introduction

Chemical restraint of southern elephant seals has been difficult to control because of initial intramuscular drug administration, variability of response (Woods *et al.* 1989), and practical difficulties associated with working under field conditions. Antagonist drugs have the potential to improve control and safety of chemical restraint by lessening central nervous system depression, improving ventilation or terminating the restraint. Their main applications are in preventing complications of prolonged chemical restraint (such as pup abandonment and hyperthermia), improving efficiency of work with the animals (such as when large numbers of animals need to be restrained each day), and in decreasing excessive levels of chemical restraint in animals breathing normally.

Drugs commonly used for chemical restraint in these animals are ketamine and diazepam, ketamine and xylazine, or tiletamine and zolazepam (Baker *et al.* 1990, Mitchell and Burton 1991). In other animals ketamine and xylazine has been antagonised using 4-aminopyridine (a releaser of acetylcholine and other neurotransmitters) and yohimbine (an α_2 adrenergic antagonist) (Kitzman *et al.* 1984); ketamine and diazepam with 4-aminopyridine (Agostons *et al.* 1980); and tiletamine and zolazepam with sarmazenil (a benzodiazepine antagonist) and doxapram (an analeptic) (Hatch *et al.* 1988). However, there are no reports of the use of antagonists in southern elephant seals and few in other seals. Cawthorn (1989) used yohimbine to antagonise ketamine and xylazine chemical restraint in New Zealand sea lions (*Phocarctos hookeri*) but found that it caused tremors and convulsions. Gales (1989) suggested that this was because yohimbine was primarily antagonising xylazine, which was then failing to mask the side effects of ketamine. Doxapram has been used as a respiratory stimulant (Parry *et al.* 1981, Cornell and Antrim 1987, Baker *et al.* 1988) but it has been suggested that it will also speed recovery from narcosis in grey seals (*Halichoerus grypus*; Parry *et al.* 1981) and the walrus (*Odobenius rosmarus*; Cornell and Antrim 1987), and results of another trial (Chapter 11) suggested that it could be useful for this purpose.

This study examined the effectiveness of antagonists in uncomplicated episodes of chemical restraint.

Materials and methods

Ninety six healthy, mature, premoulting females of median 2.50 m snout-tail length (range = 2.10 - 2.90 m) and 369 kg body weight (range = 215 - 610 kg) were selected for trials. They were divided into 4 anaesthetic groups, and given nominal doses of either 100:1 ketamine: diazepam (100:1 ratio of

ketamine to diazepam; ketamine = 3.0 mg/kg, diazepam = 0.03 mg/kg), 50:1 ketamine: diazepam (ketamine = 3.0 mg/kg, diazepam = 0.06 mg/kg), ketamine and xylazine (3.0 mg/kg, xylazine = 0.5 mg/kg), or tiletamine and zolazepam (0.5 mg/kg each drug) combined with 1.3 mg atropine sulfate (median = 0.004 mg/kg, range = 0.002 - 0.006 mg/kg, n = 96). Initially groups of 5 animals were used to test each anaesthetic antagonist against saline controls. Once sedated, animal masses were determined by weighing, or use of length-mass relationships if they were insufficiently restrained, and the actual doses of drugs administered to each group calculated (Table 12.1).

The dose of 4-aminopyridine used to antagonise 100:1 ketamine: diazepam was based on that used in humans (0.3 mg/kg; Agoston *et al.* 1980), the doses of 4-aminopyridine and yohimbine used to antagonise ketamine and xylazine on those used in horses and cattle (0.2 - 0.3 mg/kg for 4-aminopyridine and 0.075 mg/kg for yohimbine; Kitzman *et al.* 1982 and 1984, Gleed 1987), and the dose of doxapram administered to antagonise 50:1 ketamine: diazepam, ketamine and xylazine, and tiletamine and zolazepam was determined in another study (Chapter 11). The dose of sarmazenil was based upon those used to antagonise climazolam in horses (0.5 - 1 mg/kg; Ludwig *et al.* 1985) and benzodiazepines in large animals (0.25 - 5 mg/kg; Rehm and Schatzmann 1984).

Approximately 10 min after administration of the anaesthetic drugs, two 18 G, 90 mm needles (Terumo, Tokyo, Japan) were inserted into the extradural intravertebral vein. One was used for blood sampling and was located 30 to 45 cm cranial of the proximal end of the sacrum (approximately 5 or 6 vertebral body lengths). The other needle which was used for antagonist or saline was located approximately 1 to 2 intervertebral spaces cranial to the blood sampling needle. Every 5 min from 10 to 60 min blood (1 mL heparinised) was collected for blood gas and pH analysis and heart rate (beats/ min: bpm), respiratory rate (breaths/ min) and level of chemical restraint, graded from level 1 (light sedation) to level 5 (heavy immobilization) (see Footnote Table 12.3), were recorded. Definitions and techniques used for monitoring and blood gas and pH analysis were those presented in Chapter 4.

Single values for heart rate, respiratory rate, venous blood gases and pH for the 20 min period after antagonist or saline administration were generated for each animal by taking the median of values recorded at 25, 30, 35 and 40 min.

Immediately after the 20 min sample, a dose of antagonist or a similar volume of saline was slowly administered. Because of their relatively large volumes (80 to 350 mL), doxapram, sarmazenil, and their saline controls were warmed to approximately 37°C. Sarmazenil and its saline controls were infused via a drip stand, fluid bag and giving-set, run through a water bath at approximately 37°C.

Table 12.1. Doses of drugs used for chemical restraint and antagonists (median, range) administered to southern elephant seals. Anaesthetic doses were not significantly different ($P > 0.05$) between control and antagonist groups, and have been combined for clarity.

Anaesthetic	Antagonist or control	Dose of drugs used for chemical restraint (mg/kg)					Antagonist dose (mg/kg)				n
		Diazepam	Xylazine	Zolazepam	Ketamine	Tilatamine	4-Aminopyridine	Sarmazenil	Yohimbine	Doxapram	
100:1 Ketamine: diazepam	4-Aminopyridine	0.03 (0.02 - 0.05)			3.10 (2.29 - 3.93)		0.27 (0.14 - 0.41)				13
	Control										13
50:1 ketamine: diazepam	Sarmazenil	0.06 (0.05 - 0.11)			2.45 (1.97 - 4.10)			1.87 (0.93 - 3.67)			5
	Doxapram									4.68 (4.24 - 5.30)	5
	Control										10
Ketamine and xylazine	4-Aminopyridine		0.5 (0.22 - 0.79)		2.79 (1.65 - 3.93)		0.2 (0.13 - 0.21)				5
	Yohimbine								0.06 (0.05 - 0.16)		5
	Doxapram									4.19 (2.63 - 4.90)	5
	Control										15
Tiletamine and zolazepam	Sarmazenil			0.49 (0.41 - 0.93)		0.49 (0.41 - 0.93)		1.05 (0.74 - 3.98)			5
	Doxapram									4.92 (3.91 - 7.44)	5
	Control										10

Initially a fixed dose of sarmazenil was administered. However it soon became apparent that the required dose could be titrated to effect by infusing the solution until the first signs of shaking or a "dissociated look" were seen in the recipient animal. Therefore, in subsequent animals, administration of sarmazenil was stopped when this point was reached.

Data were analysed using the Mann-Whitney U test to compare two groups, and the Kruskall-Wallis H test for comparison of 3 or more groups. Differences were considered significant when $P < 0.05$.

Results

The median doses of drugs used to chemically restrain antagonist and control animals within each anaesthetic group were not significantly different (Table 12.1).

Antagonism of 100:1 ketamine: diazepam

4-Aminopyridine significantly prolonged the time to first movement forward (from 32 min to 52 min) and recovery (from 43 min to 65 min) in animals chemically restrained with 100:1 ketamine: diazepam (Table 12.2), but heart rate and respiratory rate were not significantly changed (Table 12.3).

Shaking developed in 6 of 13 animals within 10 min of administration of 4-aminopyridine, but not saline. The dose of 4-aminopyridine administered to those animals in which shaking was seen was not significantly different to that administered to those in which it was not seen. Two animals were shaking prior to 4-aminopyridine administration and this increased within min of 4-aminopyridine. One became hyperresponsive to external stimuli, showed increased muscle tone and began paddling movements with the fore-flippers. Ten mg of diazepam was administered slowly into the extradural intravertebral vein, and within 2 min muscle tone and shaking had decreased. The shaking was less marked in the other animal and gradually subsided without treatment.

One animal which received 4-aminopyridine was more subdued than the others and seemed to be blind after recovery. Both eyes appeared normal, pupils were constricted (their normal state) and the corneal response was intact, but there was no menace response and the seal behaved strangely. Rather than watching the operators and responding to vocalisations of seals around it, it remained immobile but repeatedly sniffed tussock and the air. Despite appearing reluctant to move it had disappeared approximately 5h after recovery and had presumably gone to sea; it was not sighted again. Before dosage the animal did appear to be quieter than other seals, and breathing and heart rate were normal during restraint.

Table 12.2. Time to first movement forward and recovery (median, range) for seals treated with antagonists or saline. Times to first movement forward for 50:1 ketamine: diazepam were not significantly different ($P > 0.05$) between control and antagonist groups and have been combined. (H = result of Kruskall-Wallis H test).

Anaesthetic	Antagonist or control	Time (min) to First movement forward	Time (min) to Recovery*	n
100:1 ketamine: diazepam	4-Aminopyridine	52† (34 - 72)	65† (29 - 130)	13
	Control	32 (18 - 50)	43 (18 - 53)	13
50:1 ketamine: diazepam	Sarmazenil	37 (21 - 65)	50 (45 - 60)	5
	Doxapram		34‡ (25 - 45)	5
	Control		46.5 (39 - 90)	10
			H = 7.765, P < 0.05	
Ketamine and xylazine	4-Aminopyridine	51 (33 - 59)	8h§ (43 - >16h)	4§
	Yohimbine	29 (22 - 62)	42† (24 - 80)	5
	Doxapram	24† (24 - 30)	24† (22 - 60)	5
	Control	53 (27 - 81)	90 (40 - 191)	15
		H = 9.090, P < 0.05	H = 14.386, P < 0.01	
Tiletamine and zolazepam	Sarmazenil	34 (27 - 40)	40‡ (30 - 40)	5
	Doxapram	55 (45 - 90)	50 (30 - 90)	5
	Control	44.5 (31 - 72)	50 (31 - 125)	10
		H = 9.311, P < 0.01	H = 6.603, P < 0.05	

*Level 1 chemical restraint (light sedation).
†$P < 0.01$ versus control (U test).
‡$P < 0.05$ versus control (U test).
§Only 4 values were recorded for ketamine and xylazine antagonised with 4-aminopyridine; >8h, >16h, >8h and 43 min. The animals with prolonged recovery times (> 60 minutes) left the study site, or could not be found after these times. The 5 th animal went to sea at 34 min, at level 3 restraint (heavily sedated).

Table 12.3. Heart rate, respiratory rate, blood gases and pH, and level of chemical restraint for the 20 minute period after antagonist or saline administration (median, range) for southern elephant seals chemically restrained with 4 different cyclohexamine based drug combinations. Values have been combined where they were not significantly different (Kruskall-Wallis H test: $P > 0.05$) for antagonist and control groups. (H = result of Kruskall-Wallis H tests).

Anaesthetic	Antagonist or control	Heart rate (bpm)	Respiratory rate (breaths/min)	pH or blood gas variable: pH	PvO2 (mmHg)	PvCO2 (mmHg)	HCO3- (mmol/L)	Level of chemical restraint*	n
100:1 ketamine: diazepam	4-Aminopyridine	63 (24 - 80)	6 (3 - 10)	7.35 (7.23 - 7.44)	46 (32 - 57)	50 (39 - 65)	27 (20 - 37)	3 (2 - 5)	13
	Control								13
50:1 ketamine: diazepam	Sarmazenil	60 (60 - 62)	7 (2.5 - 12)	7.32† (7.28 - 7.34)	48 (40 - 60)	49 (22 - 62)	27 (11 - 37)	2.5 (2 - 3)	5
	Doxapram	68 (65 - 81)		7.38 (7.29 - 7.43)				1‡ (1 - 2)	5
	Control	60 (52 - 67)		7.36 (7.35 - 7.43)				2 (2 - 3.5)	10
		H = 8.282, P < 0.05		H = 8.115, P < 0.05				H = 7.719, P < 0.05	
Ketamine and xylazine	4-Aminopyridine	44 (32 - 48)	5 (0 - 12)	7.26† (7.21 - 7.28)	42 (27 - 51)	51 (40 - 57)	25† (22 - 26)	3 (0.5 - 4.5)	5
	Yohimbine	60‡ (58 - 62)		7.31 (7.28 - 7.35)			27 (25 - 27)		5
	Doxapram	48 (28 - 56)		7.30 (7.24 - 7.41)			24† (23 - 27)		5
	Control	45 (36 - 60)		7.31 (7.26 - 7.40)			26 (23 - 32)		15
		H = 10.377, P < 0.05		H = 8.252, P < 0.05			H = 9.735, P < 0.05		
Tiletamine and zolazepam	Sarmazenil	62 (48 - 78)	7 (3 - 17)	7.34 (7.30 - 7.39)	52 (40 - 57)	42 (29 - 49)	23 (17 - 29)	2 (1 - 4)	5
	Doxapram			7.38‡ (7.34 - 7.41)					5
	Control			7.34 (7.29 - 7.36)					10
				H = 6.222, P < 0.05					

*1 = light sedation, 2 = moderate sedation, 3 = heavy sedation, 4 = light immobilization, 5 = heavy immobilization.

†P<0.05 versus control (U test).

‡P<0.01 versus control (U test).

Antagonism of 50:1 ketamine: diazepam

Doxapram significantly decreased time to recovery after 50:1 ketamine: diazepam (from 47 min to 34 min) while sarmazenil had little effect (Table 12.2). Level of restraint for the 20 min period after antagonist or saline was lower after doxapram compared with control values (Table 12.3).

In one animal shaking increased after sarmazenil administration and required an additional 35 mg diazepam intravenously to decrease its severity. This animal had a prolonged recovery during which it appeared to lose inhibitions and fear of the operators and other animals. Another animal which received a relatively large dose of sarmazenil (4.0 mg/kg) became temporarily blind. It first exhibited hyperreactivity and apparent hallucinatory behaviour: aimless wandering through tussock, and loss of fear. This had diminished by 2.5 h, and after 4 h it was found that the seal had no menace response and was behaving like a blind seal (caution in moving, sniffing, appearing to be listening, extension of vibrissae before movement forward). The seal was moderately sedated and heart rate and respiratory rate were regular during chemical restraint and pupils remained constricted. Twenty-four h after administration of sarmazenil there was still no menace response, however by 30 h menace response had returned and the seal appeared clinically normal.

Antagonism of ketamine and xylazine

Both yohimbine and doxapram decreased the time to recovery but 4-aminopyridine greatly increased time to recovery (Table 12.2). Time to recovery was shorter in those animals which received doxapram (24 min) than yohimbine (42 min). Shaking was associated with administration of doxapram but not yohimbine. One ketamine and xylazine animal became more responsive after doxapram administration, but relapsed by 43 min and returned to the previous level of chemical restraint.

Heart rate was faster after antagonism with yohimbine (median 60 bpm) than for the other groups (44 - 48 bpm) (Table 12.3). Hyperthermia was not seen in the 5 animals to which yohimbine had been administered but was in 4 of the 5 control animals, although ambient temperatures were similar.

Antagonism of tiletamine and zolazepam

Sarmazenil decreased the time to recovery (40 min compared with 50 min for the control) after tiletamine and zolazepam, while doxapram had little effect. One animal to which sarmazenil was administered became hyperresponsive at 33 min, but appeared to have recovered by 45 min.

The Overt Response to Antagonists (all trials)

i. 4-Aminopyridine

The overt response of animals to 4-aminopyridine was variable, however the drug usually induced shaking or prolonged time to recovery compared with control animals.

ii. Sarmazenil

The overt response of animals to sarmazenil varied but usually included fine muscle fasciculations, yawning, a "dissociated look", and/ or changes in the posture of the caudal flippers which were often lifted off the ground, the plantar surfaces tightly pressed together, digits abducted, and held in this position for up to 10 min. Often the fasciculations were so fine that they could not be seen but could be felt when the open palm was placed upon the animal's back. These responses occurred 10 to 15 min after sarmazenil administration, were not seen in the control groups and were considered to indicate the onset of benzodiazepine reversal.

iii. Doxapram

Doxapram induced transient shaking and increased muscle tone in many animals. Some animals appeared mentally alert before becoming physically alert. These animals would develop an "anxious facial expression", become hyperresponsive with increased muscle tone, "anxiously" observe the operators' movements but be unable to move forward. The head response was unpredictable at this stage.

iv. Yohimbine

Approximately 10 min after administration of yohimbine the animal would yawn, raise its head, look around and in many cases make its first movement forward. Animals also appeared much less aggressive and their responses more predictable after yohimbine than after saline or other antagonists of ketamine and xylazine.

Antagonists had little effect on respiratory rate except for a transient (< 5 min) increase in respiratory rate and/or depth in most animals after doxapram (Table 12.3).

There were few changes in pH and blood gas values after antagonist administration relative to their controls (Table 12.3). pH was lower after 4-aminopyridine in the ketamine and xylazine group and after sarmazenil in the 50:1 ketamine: diazepam group, and higher after doxapram administration in the tiletamine and zolazepam group. HCO_3^- levels were lower in the ketamine and xylazine group after 4-aminopyridine and doxapram compared with the control (Table 12.3).

Table 12.4 presents a summary of the major findings of this study.

Table 12.4. Summary of the major findings of this study on antagonism of cyclohexamine based drug combinations commonly used to restrain southern elephant seals including effective doses of antagonists, effects on time to recovery and respiration, time of onset, side effects, disadvantages and overall value.

Anaesthetic	Antagonist							
	Agent	Dose of antagonist (mg/kg)	Effect on time to recovery	Effect on respiration	Time of onset*	Side effects and disadvantages	Value for routine antagonism	Comments
100:1 ketamine: diazepam	4-Aminopyridine	0.2	Prolonged			Shaking, loss of menace response	Unreliable, possibly contraindicated	Unpredictable. May cause blindness.
50:1 ketamine: diazepam	Sarmazenil	0.5 - 1.0 or to effect	Little effect		10 - 15 min	Increased muscle tone Shaking	Unreliable and use with caution	Characteristic response: fine muscle fasciculations, dissociated look, yawning, changes in caudal flipper posture. Could be of use if higher doses or lower ratios of ketamine to diazepam are used.
						Loss of menace response		At high dose (5.0 mg/kg). May cause blindness.
	Doxapram	2.0	Decreased	Transient increase in rate and/or depth	< 2 min	Hyperresponsive Shaking, increased muscle tone	Useful for stimulating breathing	Antagonism unnecessary in most cases.
Ketamine and xylazine	4-Aminopyridine		Prolonged					
	Yohimbine	0.06	Decreased		10 min		Useful	Characteristic response: head raising, looking around, yawning, and moving off. Less thermoregulatory problems. Less aggression.
	Doxapram	2.0	Decreased	Transient increase in rate and/or depth	< 2 min	Shaking Evidence of relapse Unpredictable or dangerous head response†	Useful	More useful if improved ventilation required.
Tiletamine and zolazepam	Sarmazenil	0.5 - 1.0 or to effect	Decreased		5 - 10 min	Increased muscle tone Shaking	May be of use	Characteristic response: fine muscle fasciculations, dissociated look, yawning. Could be of use if higher doses or repeat administrations of drugs.
	Doxapram	2.0	Little effect	Transient increase in rate and/or depth	< 2 min	Hyperresponsive Shaking, increased muscle tone	Useful for stimulating breathing	Mentally alert before physically alert. Routine antagonism unnecessary in most cases.

*From time of administration.

†Head response = a graded response, based on the speed and degree of withdrawal in response to touching the top of the head and side of the muzzle.

Discussion

Antagonists were administered 20 min after the anaesthetic because most routine procedures performed in these animals could be completed in this time. Also, arousal from ketamine and diazepam, or tiletamine and zolazepam combinations, at the dosages used, usually occurs after 40 to 50 min and could confuse the results if antagonist was given any later. It has also been suggested that if ketamine and xylazine is antagonised within 20 min, central nervous system stimulation may occur (Short 1987 a).

Antagonism of 100:1 ketamine: diazepam

Prolongation of 100:1 ketamine: diazepam chemical restraint by 4-aminopyridine was unexpected demonstrating the need for controlled studies of drug usage in all species. Neither we, nor the manufacturer of 4-aminopyridine (Parnell Laboratories), are aware of any reports of prolongation of sedative or anaesthetic drug effects by 4-aminopyridine in any species. The induction of shaking by 4-aminopyridine may have been related to its neuroexcitatory actions (Sohn and Uges 1981). Although these effects are thought to be dose related the shaking seen in this study did not appear to be related to dose of 4-aminopyridine. Given the findings of this study, the normally rapid recovery from 100:1 ketamine: diazepam, the tendency for animals chemically restrained with 100:1 ketamine: diazepam to have increased muscle tone, and the relatively low levels of chemical restraint required for handling, 4-aminopyridine seems to be inappropriate for antagonism of 100:1 ketamine: diazepam in these animals and to be contraindicated.

Sarmazenil was not administered to the 100:1 ketamine: diazepam group as diazepam appeared to have minimal effect on recovery time and there was concern that sarmazenil could induce the blindness associated with its use to antagonise 50:1 ketamine: diazepam.

Antagonism of 50:1 ketamine: diazepam

Doxapram, because it caused arousal, decreased time to recovery, and produced a transient increase in respiratory rate, appeared the best of the antagonists used to reverse 50:1 ketamine: diazepam. 4-aminopyridine was not administered to the 50:1 ketamine: diazepam group because of the adverse side effects associated with its use after 100:1 ketamine: diazepam.

Antagonism of ketamine and xylazine

Yohimbine was a useful drug for antagonising the effects of ketamine and xylazine as shaking was absent and there was a reduced incidence of thermoregulatory problems and lessened aggression. These properties could prevent hyperthermia on hot days and make monitoring safer for the operator (an important consideration). The increase in heart rate caused by yohimbine was considered useful but could be dangerous in an apnoeic animal because of the increase in myocardial oxygen consumption.

The ability to antagonise ketamine and xylazine improves the usefulness of this combination relative to other cyclohexamine based drug combinations.

The doses of yohimbine used to antagonise ketamine and xylazine in this study (0.06 mg/kg) were lower than those which were used to antagonise ketamine and xylazine or xylazine alone in New Zealand sea lions (approximately 0.25 mg/kg; M. Cawthorn personal communication). This may explain the lack of shaking after yohimbine in the present study. The animals in Cawthorn's study received approximately twice as much xylazine as animals in the present study and recovered more rapidly (usually within 3 min of yohimbine administration) which might indicate that the dose of yohimbine used in the present study could be increased to cause more rapid arousal. However, until dose-response studies are performed, to avoid shaking associated with unmasking of ketamine, it may be wise to administer the 0.06 mg/kg dose of yohimbine repeatedly to effect rather than give a single large dose.

Doxapram also shortened the time to recovery of ketamine and xylazine animals. However, there were undesirable side effects (including shaking, unpredictable head response, and relapse) which have been reported previously (Hatch 1983). Doxapram may be useful where an effect upon respiratory rate is required, but its effects were transient and it may need to be repeatedly administered. There is evidence to suggest that in some species combinations of 4-aminopyridine, yohimbine and doxapram may antagonise the effects of ketamine and/ or xylazine more effectively than a single agent (Kitzman *et al.* 1982, Zahner *et al.* 1984), but other studies do not support this (Wallner 1982, Kitzman *et al.* 1984, Hatch *et al.* 1985). The present study indicates that 4-aminopyridine has a depressant effect upon these animals.

Large doses of doxapram (4 mg/kg) have been associated with hypotension in other animals (Soma and Kenny 1967), and repeated administration of lower doses (for example 2 mg/kg) may be preferable to a single large dose. This may also decrease the incidence of side effects seen after the larger dose (5 mg/kg) used in this tudy.

Antagonism of tiletamine and zolazepam

The apparent lack of effect of doxapram on recovery after tiletamine and zolazepam was surprising given its usefulness in some other animals (Hatch *et al.* 1988). This may have been due to the relatively rapid normal recovery from tiletamine and zolazepam, however the normally rapid recovery after 50:1 ketamine: diazepam was hastened by doxapram. The transient effects of doxapram on respiratory rate and depth when used to antagonise tiletamine and zolazepam were however useful.

The onset of fine muscle fasciculations and "dissociated look" after sarmazenil administration also suggests that it did appear to antagonise some of the effects of the zolazepam in tiletamine and zolazepam and the diazepam in the 50:1 ketamine: diazepam trial group. However the times to first

movement forward were unaffected by sarmazenil compared with their respective control groups (Table 12.2) which might indicate that this variable is controlled primarily by the tilatamine or ketamine in the combinations rather than the zolazepam or diazepam.

Although tiletamine and zolazepam and 50:1 ketamine: diazepam appeared to be antagonised by sarmazenil and doxapram, respectively, the low levels of chemical restraint and rapid recoveries normally seen with these drug combinations mean that routine antagonism is not indicated. Antagonism may be more useful in situations where animals are more deeply anaesthetised, recovery is prolonged, or if more benzodiazepine is used.

The two episodes of blindness seen during this study (one with 4-aminopyridine with sarmazenil) were of concern. The menace response of the 50:1 ketamine: diazepam animal which received sarmazenil and became blind was intact prior to 50:1 ketamine: diazepam administration indicating that the drugs were responsible for the loss of vision. It is unlikely that the animal given 100:1 ketamine: diazepam and 4-aminopyridine was blind before chemical restraint since, although blind seals are sometimes found (Sorensen 1950, King 1983, Lavigne and Kovacs 1988), in our experience southern elephant seals which lose vision do so because of obvious trauma and this animal's eyes appeared structurally normal.

The manufacturers of 4-aminopyridine and sarmazenil have received no other reports of blindness resulting from administration of these drugs and there appear to be no reports in the literature of this effect (Parnell and Roche, respectively personal communication). Both manufacturers stated that the loss of menace response was unlikely to be related to 4-aminopyridine or sarmazenil administration but was more likely to be a result of residual effects of the anaesthetic used in conjunction with the benzodiazepine. Assessment of vision can be difficult when the menace response is lost. We have found slowed, or absent, menace responses in some animals which appeared to be hallucinating after administration of tiletamine and zolazepam or ketamine combined with diazepam. However, animals have usually fully recovered within 2 h of drug administration; 8 h and 30 h seem long periods for this effect to be apparent if it is due to the cyclohexamine component of the combinations.

One of the more common causes of post-anaesthetic blindness is cortical damage resulting from reduced brain perfusion or anoxia during anaesthesia, but this seems an unlikely cause given the normal heart rate and respiratory rate. We have also never seen blindness in southern elephant seals which have recovered from what appeared to be severely anoxic states (cyanotic mucous membranes, prolonged apnoea, dramatic changes in venous blood gas values and pupillary dilation possibly associated with anoxia) or in apnoeic animals whose heart rates have dropped to < 4 beats/ min. Although still unexplained, blindness is highly undesirable and the combinations of drugs in which it was seen should be avoided.

This trial was limited to drugs which were considered to have the greatest potential for use. Other antagonists are available so there is scope for further work with these drugs, dose-response studies, optimising agonist/ antagonist ratios, and combinations of antagonists. However, until these trials are performed the doses of drugs presented here could be used as a guide. Specifically, ketamine and xylazine can be antagonised with 0.06 mg/kg yohimbine, and for circumstances during which an improvement in ventilation and arousal is required, 2.0 mg/kg doxapram can be used and repeated as necessary. Though sarmazenil appeared useful for antagonism of tiletamine and zolazepam at a dose of 1.0 mg/kg, the apparent blindness associated with its administration to 50:1 ketamine: diazepam animals suggest that its use should be approached with caution until dose-response studies are performed. Similarly, until further studies are performed the use of 4-AP for antagonism of 100:1 ketamine:diazepam should also be approached with caution.

Finally, the use of antagonists should be put into perspective. Given our current knowledge, their real benefit is during routine work when animals are breathing normally, procedures have been completed and arousal is required. The pressure and rate of flow in the extradural intravertebral vein may be low, as part of the adaptations to anoxia during diving, and in apnoeic animals drugs administered by this route may be ineffective (Baker and Gatesman 1985; Chapter 11). For this reason antagonists administered by this route cannot be relied upon to treat life threatening complications associated with apnoea during chemical restraint and their use at these times may be contraindicated (Chapter 11 and 13).

Chapter 13: Apnoea during chemical restraint

Introduction

Apnoea has been reported as one of the most common side effects of pinniped anaesthesia (Gales 1989) yet there is little information regarding its cause. Drug over-dose and fright have been implicated (Backhouse 1964, Gales and Burton 1987a).

Mitchell and Burton (1991) discussed fatalities which occurred during chemical restraint of elephant seals injected with various cyclohexamine based drug combinations. In several cases death was associated with prolonged apnoea. However, prolonged apnoea is normal when seals dive. Southern elephant seals dive for periods of up to two hours (Hindell *et al.* 1992) and apnoea lasting up to 40 min has been recorded for elephant seals on land (Kenny 1979, Huntley 1984). Mitchell and Burton (1991) considered that apnoea during anaesthesia did not necessarily indicate a problem, since many elephant seals have undergone prolonged apnoea during anaesthesia without ill effects (Castellini *et al.* 1986, Antonelis *et al.* 1987).

However, apnoea has been associated with deaths under anaesthesia and two explanations have been proposed. The full physiological response associated with the apnoea of sleep or diving may not have been elicited, resulting in the early development of anaerobic conditions and death within the first hour of anaesthesia (Mitchell and Burton 1991). An alternative hypothesis was proposed by Backhouse (1964), that some of the anaesthetic may be pooled in venous sinuses during "diving" apnoea and not be metabolized; when the animal "surfaces" to breath, the anaesthetic agent is released and enters the brain to cause a relapse to deeper anaesthesia.

There are also few guide-lines in the literature on if and when apnoea should be considered a problem and treatment instigated (Mitchell and Burton 1991). The theoretical aerobic dive limit has been defined as the longest breath-hold possible without any increase in blood lactate during or after the dive, and is dependent on the available oxygen stores, oxygen consumption rate, degree of peripheral vasoconstriction, and rate of lactic acid production and consumption (Kooyman 1989a). It was hypothesised that an estimated aerobic dive limit could be used as an indicator of when breathing should recommence in apnoeic animals, and therefore the relationship between apnoea and estimated aerobic dive limit was examined.

The apparent conflict in the literature and lack of guide-lines prompted us to perform a study, the aims of which were to (1) examine possible initiating causes of apnoea, (2) examine anaesthetic drug pharmacokinetics during apnoea, and (3) develop guide-lines for prevention and treatment of apnoea during chemical restraint.

Materials and Methods

General

The animals and general methods used in this study were those used in Chapter 6 (n = 225). The study was performed opportunistically on animals being restrained for other purposes. Apnoea was defined as breath-holding for > 1 min, and prolonged apnoea as breath-holding for > 10 min. Apnoea was treated after 1 min by stimulating the animal (touching its face, manipulating its caudal flukes, or rolling it from side to side).

Three aspects of apnoea during anaesthesia were studied.

Initiating factors

i. Dose and level of restraint

The dose of ketamine was compared in animals which became apnoeic (n = 55) and those which did not (n = 81). The incidence of apnoea was compared in animals at low levels of restraint (< level 5) and those at greater levels of restraint (≥ level 5). Only animals which did not receive any additional drugs or other treatment, such as gastric lavage, were included in the comparison (n = 136).

ii. Size

The incidence of apnoea was compared in small (< 1000 kg) and large (≥ 1000 kg) animals which did not receive any additional drugs or other treatment (n = 136).

iii. Additional drug administration

A comparison was made between the incidence of apnoea or breathing difficulties in animals which received additional intravenous ketamine (n = 25) with those that did not receive any additional drugs or other treatment (n = 136).

iv. Gastric lavage

The incidence of apnoea, or breathing difficulties, was recorded in animals during and immediately after gastric lavage (n = 16) and a comparison made with those which were not lavaged and did not receive any additional drugs or other treatments (n = 136).

v. Level of arousal

To examine the relationship between fright and apnoea, the incidence of apnoea was compared in animals which were sleeping prior to injection with those which were awake; and after injection by comparing the incidence of apnoea in relaxed animals with the incidence in disturbed or aggressive animals (considered to be frightened). Only animals which did not receive any additional drugs or other treatment were included in the comparison (n = 136).

Apnoea and the estimated aerobic dive limit

All 225 animals restrained with 100:1 ketamine: diazepam (Chapter 6) were used for this part of the study. The total duration of apnoea observed was compared with the estimated aerobic dive limit, calculated for each animal using the relationship presented by Hindell *et al.* (1992):

$$\text{Aerobic dive limit (min)} = (\text{lean body mass} * TO_2) / \text{RMR (Kooyman 1989 a)}$$

where TO_2 (total available oxygen) = 0.079 L/kg (Kooyman 1989 a), and RMR (resting metabolic rate) = 0.0113 (lean body mass$^{0.75}$) (L O_2/min; Schmidt-Nielsen 1983). The method used for determination of lean body mass has been presented in Chapter 7 and Appendix V.

Ketamine pharmacokinetics during apnoea

i. Animals

The level of ketamine in plasma was measured in 8 pre-moult female southern elephant seals. Seven were restrained with 100:1 ketamine: diazepam (Chapter 6), and one with ketamine and xylazine.

These seals were divided into 4 groups of 2 based on the duration of apnoea: (1) no apnoea, (2) apnoea of < 10 min duration, (3) apnoea > aerobic dive limit and survived, and (4) apnoea > aerobic dive limit and died.

ii. Samples

In animals breathing normally, blood samples were taken from the extradural intravertebral vein at 5 min intervals until 30 min, then at 10 min intervals until the level of restraint was too low for sampling. This sampling protocol was also followed in apnoeic animals, however in animals apnoeic for greater than their estimated aerobic dive limit samples were taken when possible as procedures such as intubation and ventilation often prevented sampling. In one of the seals which died, the concentration of ketamine in the hepatic sinus just after death was also measured. Sample collection and preparation was the same as that presented in Chapter 7.

iii. Analysis

The technique for analysis has been presented in Chapter 7. Two replicate extracts of each sample were analysed and the average value determined. Because some animals received drugs other than ketamine, gas chromatography - mass spectroscopy was used to check for interfering peaks, of which none were found.

In the two animals which died, packed cell volume was also measured in each of the blood samples taken for pharmacokinetic analysis.

Data analysis

Groups were compared using the Mann-Whitney U test. Relationships were examined using Spearman rank order correlation (r_S) and comparison of frequencies using χ^2 analysis. Differences were considered significant when $P < 0.05$.

Results

General

Of the animals which did not receive any additional drugs or other treatment (n = 136), a median of one episode (range = 1-3) of 6 min apnoea (range = 1 - 52 min) was seen in 55 (40%), and evidence of upper respiratory tract obstruction, difficulty breathing or shallow respiration in 7 (5%).

Initiating factors

i. Dose and level of restraint

The median dose of ketamine administered to animals which became apnoeic was not significantly different to that administered to those which did not (2.91 mg/kg, range = 1.19 - 8.42 mg/kg, n = 136) (U = 5085.5, P > 0.4). The incidence of apnoea was higher in animals at levels of chemical restraint ≥ 5 (74%) compared with animals at lower levels of restraint (36%) (Table 13.1).

ii. Size

Apnoea or breathing difficulties were more commonly seen in smaller animals (69% of those < 1000 kg) compared with larger animals (21%) (Table 13.1).

iii. Additional drug administration

Transient apnoea of approximately 4 min (range = 1 - 11 min) duration occurred in 7 of the 25 animals (28%) which received additional intravenous ketamine, and breathing slowed or became more laboured in another 3 animals. Apnoea was also seen on 2 of the 5 occasions after additional drug had been administered intramuscularly. The proportion of animals which became of apnoeic or had difficulty breathing after additional ketamine (40%) was the same as the proportion which became apnoeic after a single intramuscular dose of 100:1 ketamine: diazepam and had not received any other drugs or treatment (40%) (Table 13.1).

iv. Gastric lavage

Apnoea for the duration of lavage was seen in 9 of 16 animals (56%) and evidence of upper respiratory tract obstruction, difficulty breathing or shallow breathing was seen in 5 others. The incidence of apnoea or breathing difficulties was greater in lavaged animals (88%) than in animals which were not lavaged and had not received other or additional drugs (40%) (Table 13.1). Once lavage was completed breathing returned to normal in all but one animal. This animal did not commence breathing and was euthanased (see below).

Table 13.1. Factors associated with apnoea in southern elephant seals restrained with 100:1 ketamine: diazepam. Of the 136 animals which did not receive additional drugs or have other procedures performed on them, 55 became apnoeic and 81 did not. Twenty five animals received additional ketamine intravenously and 16 were lavaged. The failure of figures to add up under the categories of level of restraint and size is because these values were not determined in all animals. This was usually because animals were immobilized in circumstances where determination of these values was dangerous eg when anaesthetising animals at night or in crowded harems, usually for data-logger retrieval.

	Number of animals											
	Level of Restraint*		Additional intravenous ketamine		Size†		Gastric lavage		Level of arousal			
									Prior to drugs		Immediately after drugs	
	< 5	≥ 5	Yes	No	< 1000 kg	≥ 1000 kg	Yes	No	Asleep	Awake	Relaxed	Disturbed
Apnoea	32	23	10‡§	55	44	11	14‡II	55	11	44	39	16
No apnoea	57	8	15	81	23	41	2	81	9	72	61	20
	$\chi 2 = 13.541$		$\chi 2 = 0.002$		$\chi 2 = 27.878$		$\chi 2 = 12.790$		$\chi 2 = 2.063$		$\chi 2 = 0.326$	
	$P < 0.001$		$P > 0.9$		$P < 0.001$		$P < 0.001$		$P > 0.1$		$P > 0.5$	

*Not measured in 16 animals.
†Not measured in 17 animals.
‡Apnoea or difficulty breathing.
§7 apnoeic, 3 difficulty breathing.
II9 apnoeic, 5 difficulty breathing.

v. Level of arousal

The incidence of apnoea in animals which were sleeping (55%) prior to drug administration was not significantly different to that seen in animals which were awake (38%). The incidence of apnoea was also similar for animals which were relaxed after drug administration (39%) compared with those animals which were disturbed and aggressive (44%) (Table 13.1). Aggressive animals often required larger, or additional, doses of drug to induce the required level of restraint which limited work with the animals as additional time was spent waiting for drugs to have an effect.

Apnoea and the estimated aerobic dive limit

Estimated aerobic dive limits increased with the size of the animals and ranged from 21 to 33 min for animals < 1000 kg and 34 to 52 min for animals ≥ 1000 kg (Table 13.2).

Of the 55 animals in which apnoea was seen, 52 (95%) began breathing by the time the estimatedaerobic dive limit was reached, mostly within 10 min of the onset of apnoea. In most cases in these animals pH and blood gas values remained relatively stable with pH of approximately 7.35, Pv_{O2} of approximately 40 mmHg and Pv_{CO2} < 60 mmHg. Example plots of pH, blood gas and ketamine concentration in blood taken from the extradural intravertebral vein over time for one animal which did not become apnoeic, and one apnoeic for less than 10 min, are presented in Figure 13.1A and B respectively.

Of the 3 animals which received 100:1 ketamine: diazepam and whose apnoea exceeded their estimated aerobic dive limit two survived. In these two animals pH and Pv_{O2} fell gradually during apnoea to between 6.9 and 7.0 (pH) and < 10 mmHg (Pv_{O2}), and Pv_{CO2} rose to between 80 and 100 mmHg as the duration of apnoea progressed. Once breathing commenced pH and blood gas values returned to normal over the next 20 min. An example plot of pH, blood gases and ketamine concentration in blood taken from the extradural intravertebral vein over time for one animal of these animals is presented in Figure 13.1C.

The third animal which received 100:1 ketamine: diazepam and exceeded its estimated aerobic dive limit died (Figure 13.1D; tag number 3071). The animal which received ketamine and xylazine and died also exceeded it's estimated aerobic dive limit (Figure 13.1E; tag number 3049). In both animals pH and Pv_{O2} fell gradually during apnoea and Pv_{CO2} increased, however animal 3071 received 4000 mmol/L of bicarbonate at 43 min, at which time there was a rapid increase in pH, Pv_{CO2} and HCO_3^-. This animal appeared to vomit during intubation though no vomit was observable. However on post-mortem vomit was found within the nasopharynx, trapped behind a "sphincter" made by the soft palate, base of the tongue and aryepiglottic folds, effectively sealing off the oro- from the nasopharynx. There was no vomit in other parts of the airway. In both animals the packed cell volume increased during the anaesthetic episode; in animal 3071 from 64% initially to 70% just

Table 13.2. Nominal snout-tail length (STL), mass and estimated theoretical aerobic dive limits (ADL) (median, range) calculated for different categories of southern elephant seals at different times of their life cycle. Estimated masses and ADLs for subadult males could be based on those used for females of similar size and stage of yearly cycle. The categories of animals are described in Chapter 6.

Category			STL	Mass	ADL	n
Sex	Age	Stage of yearly life cycle	(m)	(kg)	(min)	
Male and female	Yearling		2.0	200	23 (21 - 28)	32
Female		Prefast	2.5	450	29 (26 - 33)	109*
		Postfast		350		
Male	Subadult		2.0 - 3.5			
	Bachelor	Prefast	3.5	1800	36 (34 - 48)	20
		Postfast		1300		
	Harem bulls					
	I. Challenger	Prefast	4.0	2400	38 (34 - 52)	31
		Postfast		1500		
	II. Beach master	Prefast	4.5	2600	40 (36 - 47)	21
		Postfast		1800		

*Mass was not determined in 10 animals

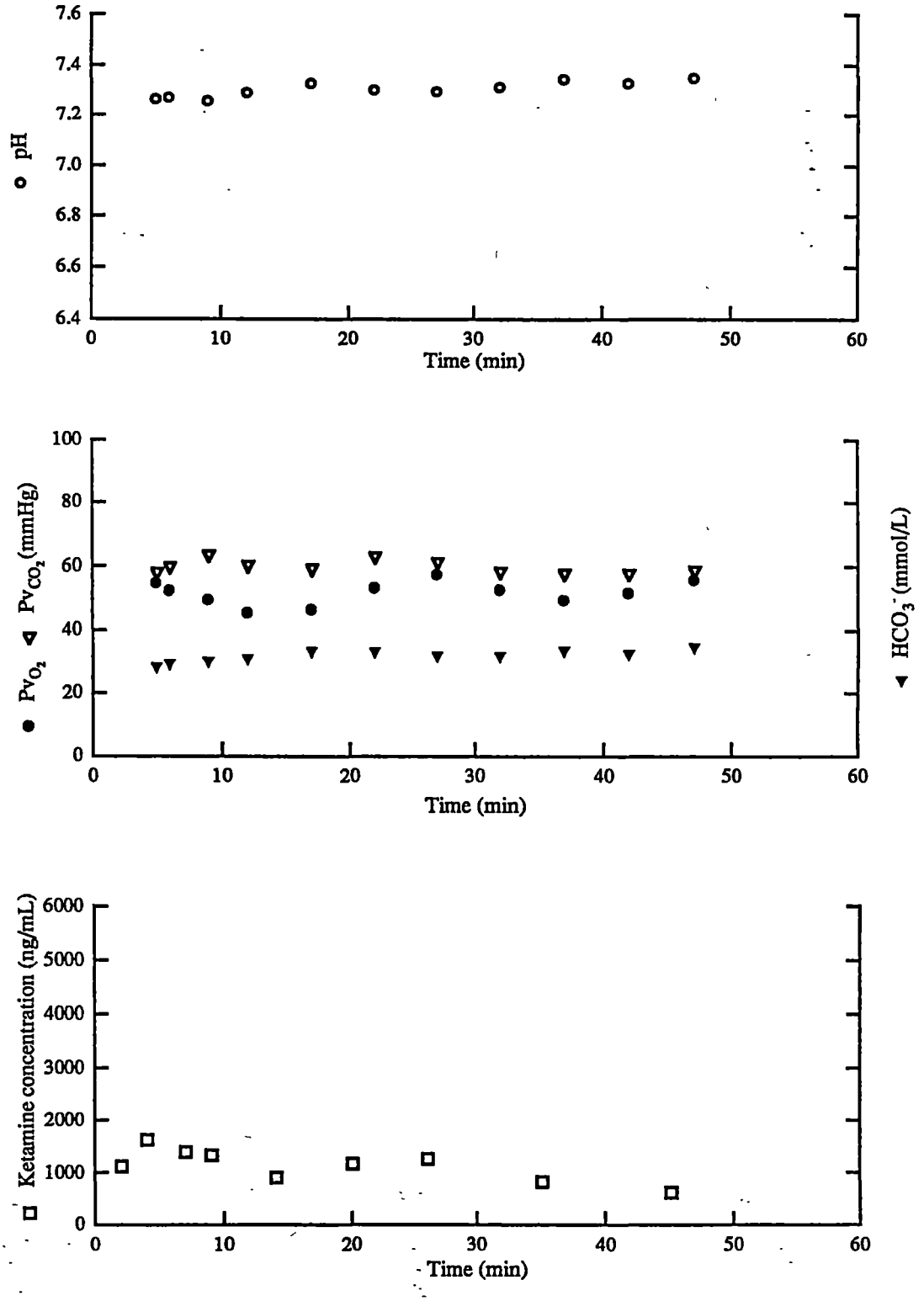


Figure 13.1A. No apnoea. An example of changes in pH, blood gas values, and the concentration of ketmine in blood collected from the extradural intravertebral vein over time in a female southern elephant seal restrained with ketamine and diazepam which did not become apnoeic.

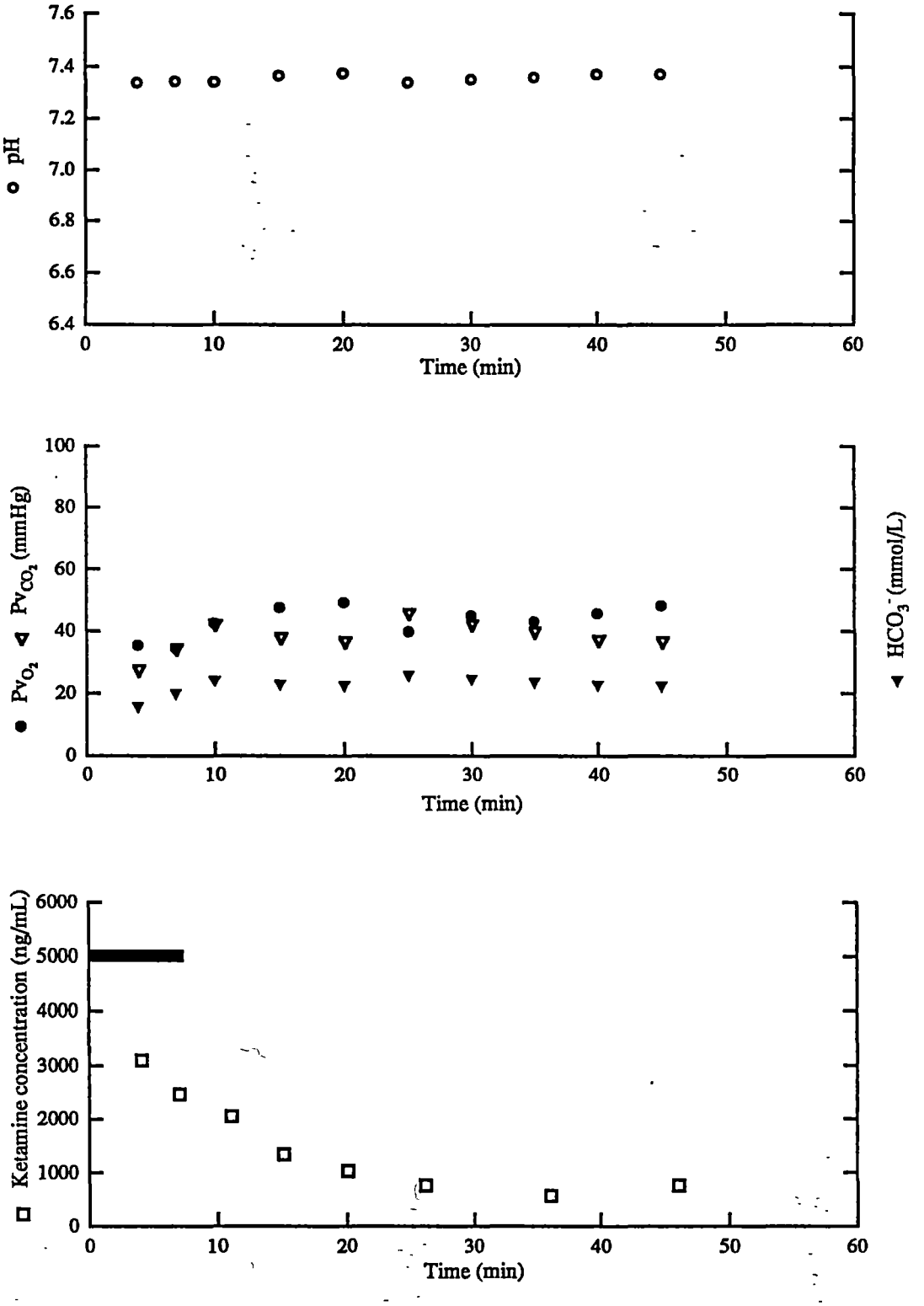


Figure 13.1B. Apnoea for < 10 min. An example of changes in pH, blood gas values, and the concentration of ketamine in blood collected from the extradural intravertebral vein over time in a female southern elephant seal restrained with ketamine and diazepam which became apnoeic for less than 10 min. The apnoeic period is represented by the solid horizontal line in the bottom graph. (The ADL estimated was 28 min).

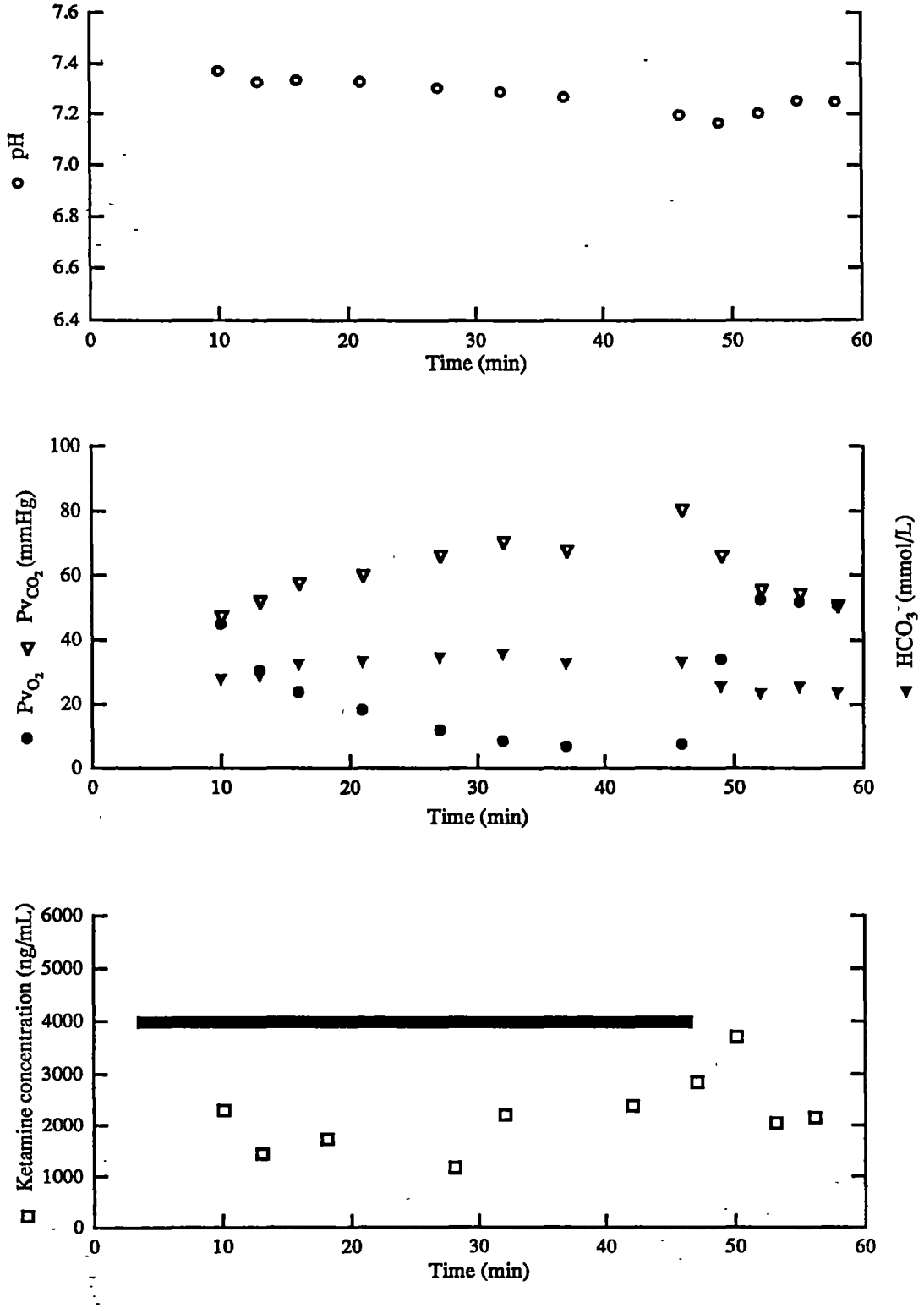


Figure 13.1C. Apnoea for > estimated ADL. An example of changes in pH, blood gas values, and the concentration of ketamine in blood collected from the extradural intravertebral vein over time in a female southern elephant seal restrained with ketamine and diazepam which became apnoeic for less than its estimated ADL (30 min) and survived. The apnoeic period is represented by the horizontal line in the bottom graph.

prior to death and in animal 3049 from 69% to 80%. (Case reports describing the time course of the anaesthetic episode for each of these animals are presented in Appendix IV.)

Ketamine pharmacokinetics during apnoea

The median concentration of ketamine in the blood at the time that the first blood sample was taken for all animals was 2.6 μg/mL and the median concentration just prior to the animals moving away was 1.2 μg/mL (Table 13.3).

In animals which breathed throughout the anaesthetic episode ketamine concentrations remained below 1.7 μg/mL (maximum level for each animal = 1.6 and 1.7 μg/mL) during the episode of restraint. The median ketamine concentration at which breathing stopped in all apnoeic animals (2.2 μg/mL, n = 8) was not significantly different to that recorded just before breathing commenced (2.8 μg/mL, n = 6) (Table 13.3).

In animals apnoeic for less than their estimated aerobic dive limit the duration of apnoea increased as concentration of ketamine at the onset of apnoea increased. However, average ketamine concentrations at the onset of apnoea were significantly higher in the 4 animals apnoeic for less than their estimated aerobic dive limit (median = 3.5 μg/mL) than in the 4 which exceeded their estimated aerobic dive limit (median = 2.2 μg/mL) ($U = 0.00$, $P < 0.05$; Table 13.3).

There was a rapid fall in the concentration of ketamine with time in one animal which became apnoeic for less than 10 min (Figure 13.1B). This animal received 3.01 mg/kg ketamine with 0.027 mg/kg diazepam, reached a maximum level of restraint of 4 in approximately 2 min, which lasted for 15 min. The animal had recovered 50 min later.

There was no clear trend in ketamine concentrations of the animals which were apnoeic for longer than their estimated aerobic dive responses and survived. However, in one animal, for which the most data were available, ketamine levels decreased until about 20 to 30 min and then increased until breathing recommenced when there was a rapid rise in ketamine level followed by a fall (Figure 13.1C). A fall in ketamine concentration after breathing commenced was also seen in the other animal which was apnoeic for longer than 30 min.

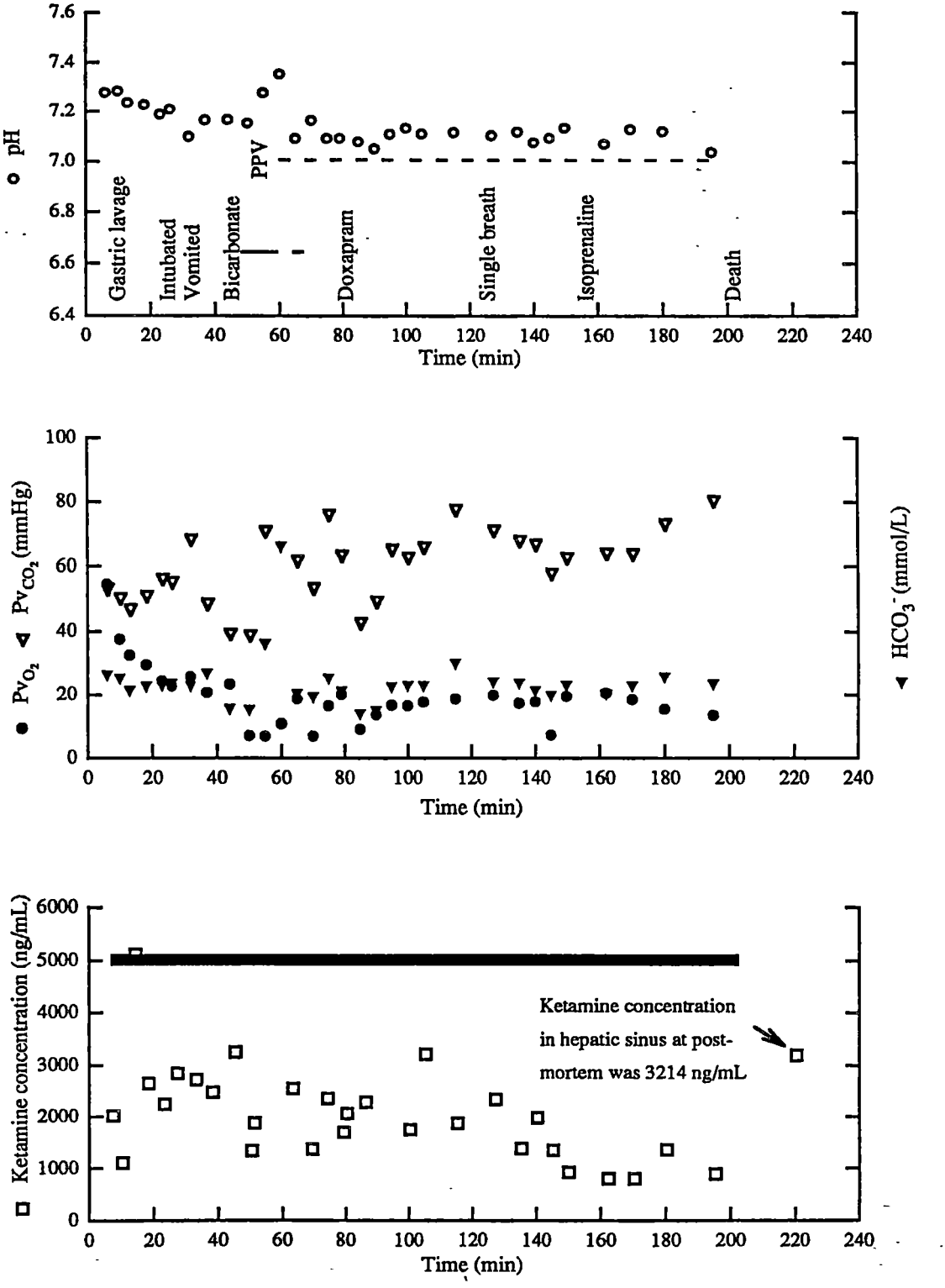


Figure 13.1D. Apnoea for > estimated ADL and died. Changes in pH, blood gas values, and the concentration of ketamine in blood collected from the extradural intravertebral vein over time in a female southern elephant seal restrained with ketamine and diazepam which became apnoeic for longer than its estimated ADL (29 min) and died. The apnoeic period is represented by the solid horizontal line in the bottom graph and the animal's tag number was **3071**). (PPV - - - - - - - - = duration of positive pressure ventilation; Bicarbonate —— - —— = duration of bicarbonate administration).

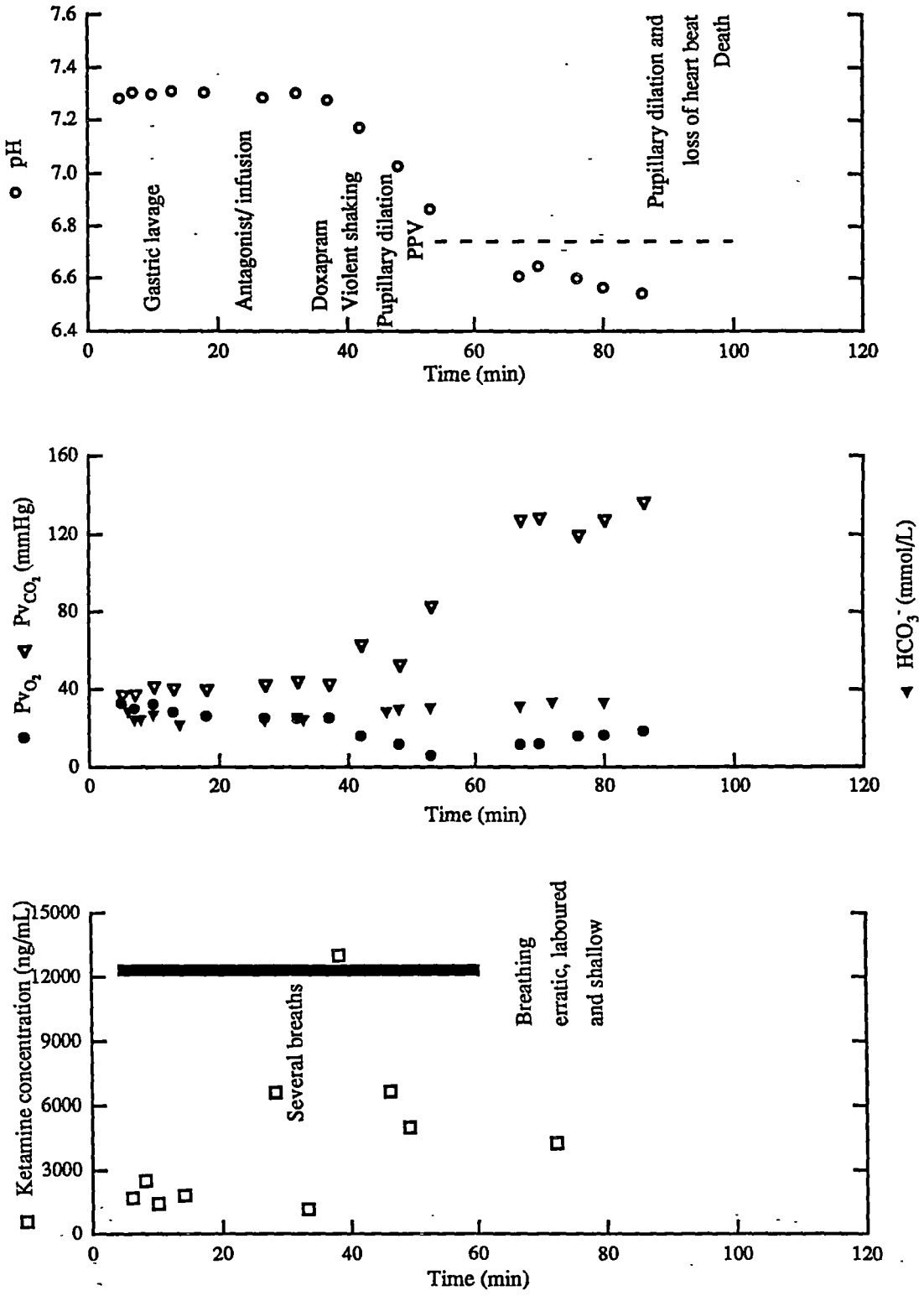


Figure 13.1E. Apnoea for > estimated ADL and died. Changes in pH, blood gas values, and the concentration of ketamine in blood collected from the extradural intravertebral vein over time in a female southern elephant seal restrained with ketamine and xylazine which became apnoeic for longer than its estimated ADL (31 min) and died. The apnoeic period is represented by the solid horizontal line in the bottom graph and the animal's tag number was **3049**). (PPV - - - - - - - - = duration of positive pressure ventilation).

Table 13.3. Ketamine concentration in plasma (average of two or median, range) taken from the extradural intravertebral vein during chemical restraint of 9 southern elephant seals chemically restrained with 100:1 ketamine: diazepam and 1 with ketamine and xylazine. The animal which received ketamine and xylazine died.

Duration of apnoea		Ketamine concentration in plasma (µg/mL)				n
		Initial	At the onset of apnoea	At the commencement of breathing	Before moving away	
Less than estimated aerobic dive limit	None	1.4	1.2	-	0.9	2
	< 10 minutes	3.0	3.0	3.0	1.1	2
	> 10 minutes	4.3	4.3	2.7	1.3	2
	All animals	3.0 (1.1 - 4.7)	3.5 (2.8 - 4.7)	2.8 (2.6 - 3.1)	1.1 (0.6 - 1.6)	6
Greater than estimated aerobic dive limit	Survived	2.2	2.2	2.8	1.6	2
	Died	2.3	1.5	-	3.0* (1.7 - 4.3)	2
	All animals	2.2 (1.8 - 2.9)	2.0 (1.3 - 2.3)	2.8† (2.4 - 3.1)	1.6† (1.1 - 2.8)	4
All animals		2.6 (1.1 - 4.7)	2.2 (1.1 - 4.7)	2.8‡ (2.4 - 3.1)	1.2§ (0.6 - 2.2)	10

*Before death.
†n = 2
‡n = 6
§n = 8

Ketamine concentrations in the apnoeic animals which exceeded their estimated aerobic dive response and died were characterised by prolonged high concentrations with large fluctuations, either increasing or decreasing over time. In animal 3071, for which most data were available, there was a rapid initial fall in ketamine concentration followed by apnoea and an increase in ketamine concentration. The concentration of ketamine in the hepatic sinus of this animal after death was 3.2 μg/mL, approximately 3 times that last recorded in the venous blood (0.9 μg/mL) (Figure 13.1D). Few values were recorded in the other animal, however ketamine concentration fell after several breaths were taken at 33 min (Figure 13.1E). (Note that the scales on the abscissae in Figures 13.1D and E differ. They are also different to those in Figures 13.1A to C).

Recommendations for treatment of apnoea

Table 13.4 presents techniques which can be used to prevent or treat apnoea. Figures 13.2 and 13.3 present an approach developed to treat apnoea and breathing difficulties in this study.

Discussion

General

Because apnoea was prevented in this study by stimulating animals to breath, the results presented need to be interpreted with caution. Apnoea did not appear to be related to dose of ketamine administered, but was more commonly associated with deeper (≥ level 5) restraint and smaller (< 1000 kg) animals. This indicates that working at low levels of restraint could be used to decrease the incidence of apnoea. However, because the dose of ketamine at which apnoea was seen (median = 2.83 mg/kg) was the same as that at which it was not seen (median = 2.75 mg/kg), and because this dose is the lowest at which intravenous access can be attained in the majority of animals (level 3 - 4 restraint; Chapter 6), prevention of apnoea at these levels by using lower drug dosage may not be possible without compromising the ability to handle the animal, and induction of apnoea should be expected as a normal occurrence during restraint. Despite its high incidence, in most cases apnoea can be prevented or treated by nociceptive stimuli to the animal or by rolling it over (see Physical techniques, Table 13.4). The higher incidence of apnoea in smaller animals was probably due to treatment bias. There are problems associated with treatment of apnoea in large animals (Chapter 11) and for this reason greater efforts were made to prevent its occurrence in them.

Table 13.4. Some techniques used to stimulate breathing or aid respiration in chemically restrained southern elephant seals, how they are performed and their usefulness.

Technique		Method	Comments
Physical	Repositioning	Ensure no pressure on trachea or chest, extend neck and position cows in sternal recumbency, bulls on their back and side.	Occasionally useful
	Tapping muzzle	Gentle tap of either side of muzzle.	Useful for stimulating breathing early in the anaesthetic episode or regularly during chemical restraint at low levels of chemical restraint (eg < 5). May also be useful at greater levels chemical restraint.
	Head manipulation	Either side of muzzle (nose in bulls) grasped firmly in both hands and head shaken side to side or lifted upwards and allowed to drop back into a normal resting position.	Use caution in bulls when tapping muzzle or manipulating head as they can appear more heavily chemically restrained than they are; similarly with ketamine and xylazine immobilized cows.
	Caudal flipper manipulation	Both caudal flippers grasped and swung from side to side. Pressure applied across the width of D1 of the caudal flukes.	Rocking can be achieved with a needle in the extradural intravertebral vein, for rolling it needs to be removed. Useful techniques for deeper levels of restraint as well as lower levels.
	Rocking	A rocking motion is induced by intermittently putting adaxial pressure on the chest. As rocking excursions become more marked the animal can be rolled over. After 1 or 2 rolls there is a pause to see if breathing will commence.	Rolling of cows required 2 to 3 people; bulls 6 to 12. Some animals cannot be rolled due to positioning.
	Rolling		Most useful into extradural intravertebral vein at low levels of chemical restraint (< 5).
	Introduction of needles	Into the extradural intravertebral vein (18 G, 90mm) or philtrum (18 G, 38mm).	Little use. Can't be sure of maintaining airway, difficult or impossible to hold open in strong cows or bulls for prolonged periods.
	Holding nares open	A finger is inserted into the nostril and pressure applied dorsally and abaxially.	Little use. Cannot be passed into nasopharynx due to ethmoidal labyrinth occluding the fundus of the nasal cavity.
	Nasopharyngeal tubes	Approximately 20 cm x 15 mm outside diameter soft plastic lubricated and slid caudally up either nostril.	Sometimes useful in light animals. These animals may cough, bite down or shake head. Dangerous in light bulls.
	Adducting arytenoids	With mouth held open (Chapter 3) a finger is slid between the adducted arytenoid cartilages.	
Chemical	Doxapram and antagonists	Chapters 11 and 12.	Only useful for stimulation of respiration in breathing animals, unreliable in apnoeic animals and may be contraindicated.
Intubation and mechanical ventilation	Intubation	Chapter 3. Check for vomit and clear from back of throat if necessary.	May exacerbate problem at depth < 5. Occasionally useful and allows positive pressure ventilation and maintainence of an airway.
	Nasopharyngeal oxygen	Chapter 3.	Useful for assisted respiration if respiratory rate is low or upper respiratory tract obstruction, or in animals too light to intubate.
	Assisted	Chapter 3.	Useful with an endotracheal tube if respiratory rate is low or there is evidence of upper respiratory tract obstruction.
	Positive pressure	Chapter 3.	Treatment of choice in animals less than 1000 kg but there are difficulties with larger animals and practical problems associated with intubation, size and use of equipment in isolated area.

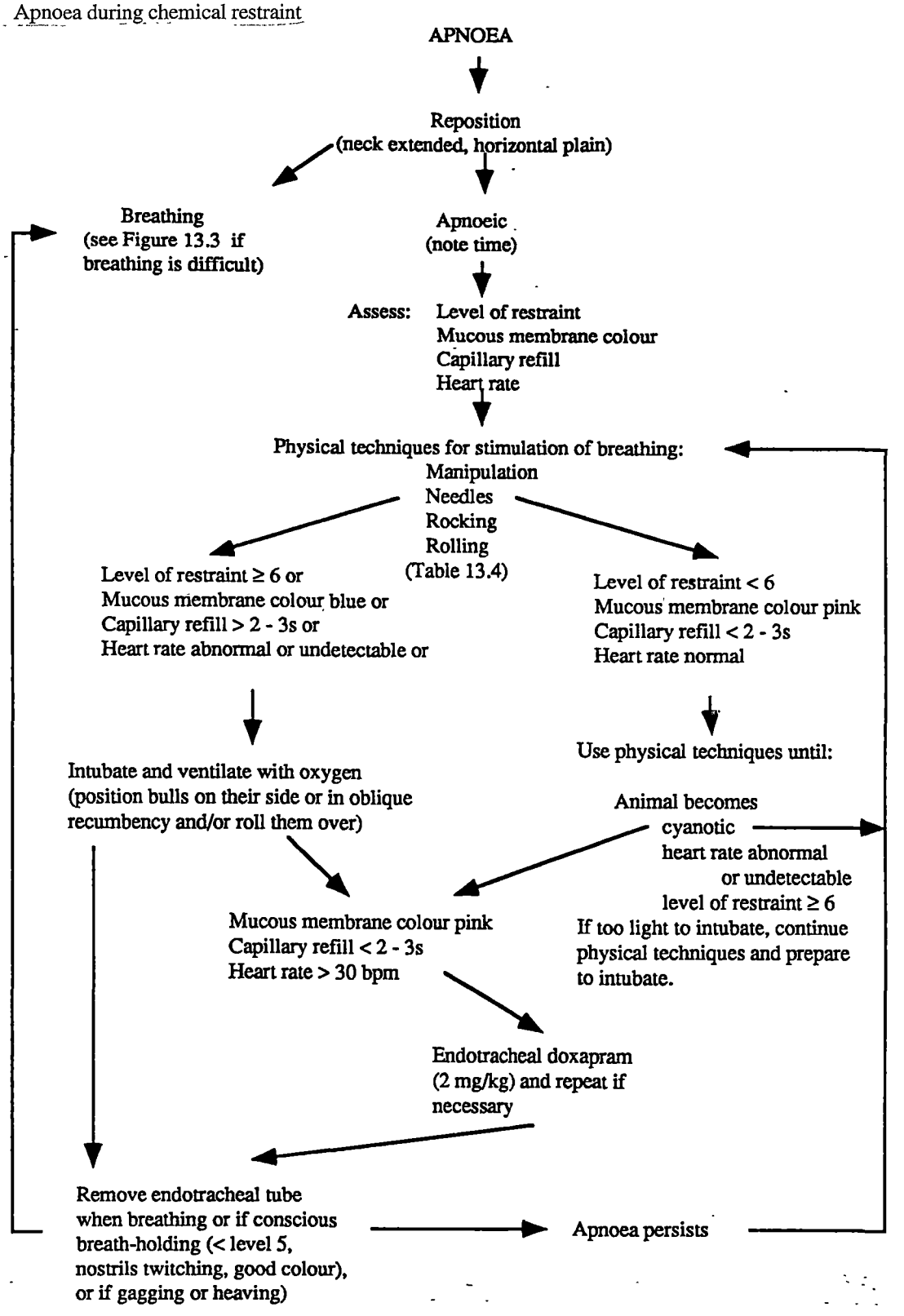


Figure 13.2 A flow chart for treatment of apnoea in southern elephant seals. Normal and abnormal values for heart rate can be found in Table 6.3, and appropriate sizes of endotracheal tube for particular categories of animal in Chapter 3 and Table VI.2. Effective ventilation of bulls may not be possible. In these animals doxapram could be administered endotracheally whilst the animal is still cyanotic (Chapter 11). (Level of chemical restrain 6 = light anaesthesia; Chapter 4).

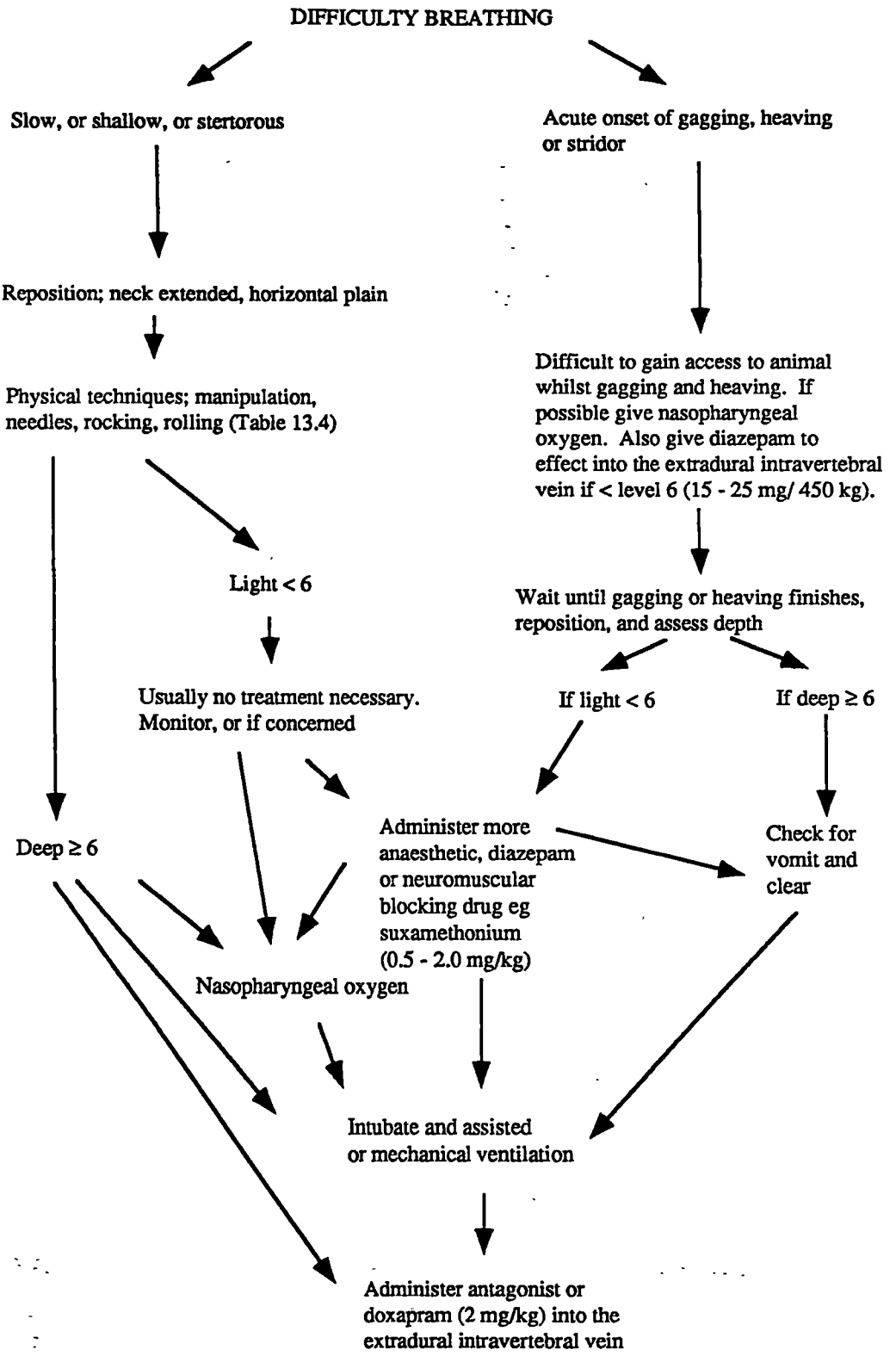


Figure 13.3. A flow chart for treatment of breathing difficulties in southern elephant seals. Doses of antagonists can be found in Chapter 11. (Level of chemical restraint 6 = light anaesthesia.) Because it can be difficult to differentiate between vomiting and laryngeal spasm due to the anatomy of the upper respiratory tract, it is recommended that examination for the presence of vomit is made if animals become apnoeic. (Level of chemical restraint 6 = light anaesthesia; Chapter 4.)

Apnoea and additional drug administration

Despite the incidence of apnoea being no greater after additional intravenous ketamine administration, apnoea was often observed and it may be expedient to administer further ketamine as repeated, small doses, slowly over 30 - 60 s to effect, rather than rapidly as a single large bolus, and to be prepared to treat any ensuing apnoea.

Apnoea and gastric lavage

Apnoea and breathing difficulties were commonly associated with gastric lavage. Once the lavage tube was removed breathing returned to normal indicating that the presence of the tube was responsible for the breathing difficulties. Therefore, if animals need to be lavaged and the duration of apnoea minimised, lavage should be carried out as rapidly as possible. Prevention of breathing by foreign bodies in the airway was also seen when intubating animals with an endotracheal tube (Chapter 11). The animals in this study were not intubated because this was considered to increase the level of restraint required and predispose to apnoea. The light levels of restraint used, tight adduction of the arytenoids and presence of a swallowing reflex would allow the airway to protect itself. HOWEVER, THE TWO ANIMALS WHICH DIED DURING THIS STUDY HAD VOMITUS IN THE UPPER AIRWAY WHICH WAS NOT VISIBLE AND FOR THIS REASON IT IS RECOMMENDED THAT AN ENDOTRACHEAL TUBE BE IN PLACE WHILST LAVAGING SEALS. If this is not possible due to insufficient restraint, the animal should be positioned head down to allow drainage of vomitus during the procedure; the gastric lavage tube should be emptied as much as possible and "kinked over" upon withdrawal to prevent fluid entering the oro- and nasopharynx; appropriate volumes of fluid used*. The airway should also be checked for the presence of fluid if this is suspected (eg if there is evidence of vomiting or regurgitation of fluid) and intubation performed.

Apnoea and level of arousal

Surprisingly, level of arousal appeared to have little effect on the incidence of apnoea. However, aggressive animals often required larger, or additional, doses of drug to induce the required level of restraint. Administration of drug to quiet, somnolent animals was therefore preferred as time was not spent waiting for additional doses of intramuscular drug to have an effect.

Apnoea and the estimated aerobic dive limit

Only three of the 55 animals which became apnoeic after a single dose of 100:1 ketamine: diazepam exceeded their estimated aerobic dive limit (5% of apnoeic animals and 2% of all animals). It is unknown if a larger number would have exceeded this limit if allowed to remain apnoeic and not stimulated to breath. It could therefore be argued that apnoea for periods less than the estimated aerobic dive limit is a normal occurrence and should not need to be treated. This is correct in the majority of cases, however some apnoeic animals will go on to exceed their estimated aerobic dive

* Three to 4 lavages of approximately 3 to 4 L each is usually adequate for cows, 2 - 3 L for yearlings, and 4 - 6 L for bulls.

limit and some of these will progress to death; others will become cyanotic and excessively deeply anaesthetised inside their estimated aerobic dive limit. Furthermore the aerobic dive limit is often predicted based on oxygen stores and availability and a predicted rate of utilization. What this utilization rate is during the highly irregular conditions of anaesthesia is impossible to guess (Fedak personal communications 1994). For these reasons other indicators, such as mucous membrane colour and degree of central nervous system depression, are probably better indicators of whether apnoea should be treated.

Ketamine pharmacokinetics during apnoea

The small sample sizes make interpretation of these results difficult. Also, ketamine concentrations in the extradural intravertebral vein may not reflect central nervous system concentrations (below). The median concentration of ketamine at which apnoea was induced (2.2 μg/mL, n = 8) was greater than the highest concentrations recorded in the two animals in which apnoea was not seen (less than 1.7 μg/mL) which implies that induction of apnoea may be dependent on ketamine concentration in the blood. However, the average concentration of ketamine at which apnoea was seen in the two animals which died was 1.5 μg/mL. This implies that other factors may be responsible for its induction and maintenance. In the animals which died gastric lavage, intubation, and vomit in the upper respiratory tract were all implicated.

The rapid fall in the concentration of ketamine with time in one animal which became apnoeic for less than 10 min (Figure 13.1B) suggest that the drugs may have been administered intravascularly. Gales and Burton (1987a) reported a fatality after suspected intravascular drug administration of 8.20 mg/kg ketamine with 0.04 mg/kg diazepam to a southern elephant seal; presumably the 3.01 mg/kg ketamine with 0.027 mg/kg diazepam administered to the animal in the present study was a safer intravascular dose. It did however, appear to induce apnoea and for this reason care should be taken to avoid initial intravascular drug administration. Blood did not flash back into the hub of the needle when testing for intravascular location of the needle prior to drug administration in this animal. Presumably the lumen of the needle was blocked with tissue or the bevel occluded by the vessel wall. If this is the case, then slow administration of drug whilst repeatedly checking for intravascular access during intramuscular drug administration may be wise. If blood flashes back into the hub of the needle or less resistance to injection is felt then administration could be delayed, the animal's response observed, and administration rate, dose of drug, or needle location adjusted accordingly.

The most striking finding in animals which underwent prolonged apnoea were the fluctuations in ketamine concentrations with time and the prolonged high concentrations of ketamine during apnoea compared with those for breathing animals. However, these data need to be interpreted with caution because (1) free drug was not measured in plasma and (2) because ketamine concentration was not measured in the brain. These data may therefore not accurately reflect concentration of free drug in the brain or plasma. It is however, interesting to speculate on possible causes for the fluctuations. There

are many factors which influence drug concentration in the blood after an intramuscular dose including rates of absorption, distribution and elimination, each of which in turn is influenced by lipid solubility of the drug, extent of binding to plasma proteins (47% for ketamine in humans; Martindale 1989) and extravascular tissue constituents, and blood flow rates through organs and tissues (Baggot 1982 a).

Seals have developed a range of physiological changes associated with diving, including bradycardia and a decrease in cardiac output and peripheral circulation (Scholander 1940, Elsner *et al.* 1966, Elsner 1969, Kooyman *et al.* 1981, Butler and Jones 1982). Metabolism is also reduced in many tissues (Kooyman *et al.* 1981). Blood returning to the heart via the caudal vena cava is restricted by the caval sphincter level with the diaphragm and pools in the inferior vena cava and hepatic sinus; blood flow to the liver decreases (Harrison and Tomlinson 1958). This system acts as a storage compartment for relatively well-oxygenated blood. In northern elephant seals approximately one fifth of the total blood volume can be contained within the inferior vena cava (Elsner 1969). In the latter portion of the dive, blood oxygen has been shown to be higher in the inferior vena cava than in arterial circulating blood (Elsner 1969). During the dive the caval sphincter opens periodically during diastole letting oxygenated blood enter the heart-brain circulation (Butler and Jones 1982). These actions conserve oxygen and glucose for the heart and central nervous system (Kooyman 1968, Robin *et al.* 1981) and have been referred to as the "dive response" (Hubbard and Poulter 1968). On land southern elephant seals have been described as showing a rhythmic variation of breathing rate characterised by some min of eupnoea followed by a period of apnoea of variable duration (Kenny 1979). There is evidence to suggest that, in the closely related northern elephant seal (*Mirounga angustirostris*), this "sleep apnoea" has physiological aspects which are similar to those seen in freely diving seals (Castellini *et al.* 1986) and it has been theorised that circulatory changes consistent with those seen during diving can occur during anaesthesia in southern elephant seals (Gales and Burton 1987 a). It is likely that some of these changes would affect anaesthetic drug kinetics.

In other animals, redistribution of ketamine from the brain to other tissues is primarily responsible for termination of its hypnotic or anaesthetic effect (White *et al.* 1982). The findings of this study suggest that clearance of ketamine decreased with apnoea, leading to prolonged high levels of ketamine in the peripheral venous blood. If the caval sphincter opens periodically during diastole (letting oxygenated blood enter the heart-brain circulation as it does in diving seals; Butler and Jones 1982), then ketamine concentrations within the central nervous system may be prolonged at high concentrations as anaesthetic laden blood is periodically released from the hepatic sinus; a possible mechanism for the prolongation of anaesthesia associated with apnoea reported by Mitchell and Burton (1991). Decreased hepatic blood flow associated with apnoea (Harrison and Tomlinson 1958), decreasing the rate of ketamine metabolism, and changes in circulation decreasing rate of distribution of drug could also contribute to this effect.

Backhouse (1964) also suggested that when apnoeic, anaesthetised animals start breathing, anaesthetic-laden blood held pooled in the hepatic sinus might rapidly be released into the heart-brain circulation increasing the level and/ or duration of restraint. Though speculative, the rapid rise in ketamine concentration at the time of return to breathing in one animal (Figure 13.1C), coupled with the relatively high concentration of ketamine found in the hepatic sinus of animal 3071 which died relative to that last recorded in its venous blood, could be interpreted as supporting this theory. The changes in blood gases in this animal ie progressive respiratory acidosis during apnoea (suggesting minimal muscle perfusion) followed by metabolic acidosis at the end of apnoea (suggesting washing out of muscle compartment) could also be interpreted as supporting this suggestion.

However, as previously stated, without levels of free drug in the brain and hepatic sinus being measured these data need to be interpreted with caution and inferences of central arterial blood gas values based on peripheral venous values are very tenuous (Chapter 3). Sampling from the brain and hepatic sinus during apnoea, and measurement of free drug, would help clarify these points, although the data do indicate that apnoea has an effect on ketamine kinetics. For this reason it is important that, until further knowledge is gained, apnoea should be avoided if possible. It also suggests that the use of anaesthetics, anaesthetic antagonists and analeptics during apnoea may be contraindicated, their kinetics being affected and concentrations possibly becoming excessive when circulation returns.

Other factors which could be influencing ketamine concentrations include changes in the absorption rate of ketamine from muscle, associated with circulatory and perfusion changes during apnoea. Ketamine has a pKa of 7.5 which indicates that the concentration of free drug in plasma could also be effected by changes in blood pH; ionization increasing as the pH of the plasma decreases and drug theoretically becoming trapped within the central compartment, decreasing distribution and clearance as elimination increasingly relies upon renal excretion, also possibly decreased due to decreased glomerular filtration rate associated with apnoea. The changes in packed cell volume in the animals which died could also affect ketamine concentrations by altering volume of distribution.

The direction of flow in the extradural intravertebral vein could also affect ketamine kinetics. Ronald *et al.* (1977) measured the velocity and direction of blood flow in the extradural intravertebral vein of the harp seal (*Pagophilus groenlandicus*). Blood flow varied directly with increasing and decreasing heart rates. Furthermore, when heart rate was above 50 beats/min, dye injected into the extradural intravertebral vein at, and posterior to the fourth thoracic vertebra, passed anteriorly. Between 40 and 50 beats/min, dye boluses in the thoracic and lumbar regions were observed to stop, and then sluggishly reverse direction as the heart rate further decreased, moving posteriorly. With acceleration of the heart, the blood immediately flowed anteriorly. There also appears to be some control of the ratio of oxygenated blood to deoxygenated blood entering the heart in the bradycardic seal (Ronald *et al.* 1977). In one case, early in the dive, two heart beats were observed by these authors without the emission of boluses of blood from the caval sphincter. This would have the effect of reducing the

proportion of relatively well oxygenated, venous, hepatic sinus blood sent to the brain and affecting ketamine concentrations. These mechanisms may offer an explanation for the fluctuations in ketamine concentrations during prolonged apnoea in the animals in the present study. Interpretation of anaesthetic drug kinetics in these animals may therefore require simultaneous determination of blood flow (direction and rate) at multiple sampling sites. Though speculative, it is possible that changes in flow occured at approximately 30 min in the animal presented in Figure 13.1C.

Both animals which died received bicarbonate (Appendix IV) which could also have contributed to trapping of ionized ketamine within the central nervous system if "paradoxical" intracellular brain acidosis occurred (Shapiro *et al.* 1989). Sodium bicarbonate treats metabolic not respiratory acidosis. Bicarbonate was administered to these animals as we had found that when it was administered previously to an apnoeic animal, the animal commenced breathing almost immediately. We theorised that this may have been because of an increase in arterial CO_2, which has been associated with its administration in other animals (Shapiro *et al.* 1989), stimulating the seal's respiratory centre which is relatively insensitive to CO_2 (Hubbard 1969). The theory behind its use was thus to increase arterial CO_2 to stimulate breathing. Despite the potential for "paradoxical" intracellular brain acidosis (Shapiro *et al.* 1989), if this was effective it had the potential to be very useful because it would mean that breathing could be stimulated at will and intubation and positive pressure ventilation, which might be difficult for people with little experience with anaesthetics or in some situations, may not be necessary. The administration of bicarbonate to both animals did not stimulate breathing and may have induced intracellular brain acidosis, possibly increasing ketamine trapping. Its use as a respiratory stimulant is thus probably contraindicated. If neutralization of acid is required, for example in treatment of cardiac arrest, therapy with Carbicarb (a recently formulated buffer that is a combination of 0.33 M sodium carbonate and 0.33 M sodium bicarbonate), which results in systemic alkalinization without major changes in arterial CO_2 and intracellular brain alkalinisation (Shapiro *et al.* 1989), may be useful. However, this treatment may only be useful when breathing commences and metabolic acidosis occurs: intubation and ventilation with O_2 is the treatment of choice for respiratory acidosis.

Summary and conclusions

Despite their ability to survive apnoea during chemical restraint, apnoea should **ALWAYS** be considered abnormal and **ANY** change in breathing pattern is cause for concern. The ability to intubate and ventilate animals should always be available when using chemical restraint.

Chapter 14: Synthesis and conclusions

This study set out to test the unifying hypothesis that:

the safety to the animals of the anaesthetic episode could be improved by using the most appropriate drugs, more accurately refining dose rates and developing techniques for the early recognition and treatment of complications.

Previously reported fatality rates in southern elephant seals anaesthetised with various drug combinations ranged from up to 3% for smaller animals (Chapter 8) to up to 50% for larger animals (Chapter 2). The techniques developed in this study improved the safety of anaesthetic use, with fatality rates of less than 1% for both small and large seals restrained with a variety of drugs.

Use of a standardised system of monitoring by a dedicated operator allowed early recognition and thus prevention or treatment of complications. It also provided a common vocabulary with which to discuss and compare particular anaesthetic episodes, anaesthetics and other findings. Future studies on chemical restraint of southern elephant seals should use a standardised system of monitoring to allow comparisons to be made between drug doses and types.

Monitoring pH and blood gas changes in the extradural intravertebral vein was a rapid and useful technique; however, the values determined may not accurately reflect changes occurring centrally and for this reason comparative studies of venous with arterial pH and blood gas values are required. This comparison was attempted but without success because of the relatively low levels and short durations of restraint and the cold working conditions. Greater restraint of animals, for longer periods, and moving them to enclosed, warmer areas for catheter placement and sampling would be required. For this purpose maintenance on gas, or the use of small, incremental doses of ketamine administered intravenously after midazolam and pethidine sedation, may be useful.

Although it gave useful information, pH and blood gas analysis required some expertise and more simple, practical techniques are required for routine assessment of central blood gas and pH values. An alternative technique, which needs to be assessed, is pulse oximetry of the tongue. Under field conditions the status of the respiratory and cardiovascular systems can be determined by monitoring breathing, heart rate, mucous membrane colour and capillary refill.

Given the length-mass relationships developed (Chapter 5) and nominal dosages for different categories of animals at different stages of their life and yearly cycles (Chapter 6), a safe dose of ketamine and diazepam to chemically restrain southern elephant seals can be determined. Estimation of mass, however, remains a problem for other stages of the animals' life cycle. Photogrametry may be an

alternative. However, the accuracy improves with experience of a particular category and in most cases operators can quickly estimate mass to within about 5% of the actual value. Until this stage is reached it would be wise to under-dose animals.

Despite the success of the doses used to restrain bulls (Chapter 6), these animals still represent a poor risk group, primarily because there are no techniques to ventilate them effectively (Chapter 11). Until such time as these techniques become available it may be wise to approach bull anaesthesia with care and be rigorous in attempting to prevent apnoea. Size also appeared to affect some variables associated with monitoring and the effect of size on respiratory exchange, various physiological parameters and drug dosage requirements during anaesthesia needs to be examined.

All drug combinations assessed could be used safely to restrain southern elephant seals and choice of an anaesthetic agent is probably at the discretion of the particular operator. Tiletamine and zolazepam offered some advantages over other commonly used combinations; however, this combination needs to be used with caution until its safety margin can be determined using dose-response studies(Chapter 8). Until the effect of tiletamine and zolazepam is examined in larger seals it may be wise for inexperienced operators to use the doses of ketamine and diazepam presented in this study as they are known to be safe and effective.

The ability to antagonise ketamine and xylazine improved its usefulness relative to other commonly used agents (Chapter 12). Similarly, because of the ability to antagonise pethidine, combinations based on this drug were also very useful, relatively safe and versatile. However, they had the disadvantages of slow onset of maximum effect and large dose volumes which could preclude their use under field conditions (Chapter 9). Medetomidine combined with ketamine appeared to offer few advantages over xylazine combined with ketamine (Chapter 10). Because of the improvement in control of the anaesthetic episode, future studies examining the use of antagonisable drug combinations are indicated; specifically to develop techniques for restraint in situations where gaseous anaesthesia cannot be used or animals cannot be ventilated.

Despite the problems, elephant seal anaesthesia becomes relatively straight forward with experience. Most investigators have achieved reasonable results with each of the drug combinations which they have used. The real difficulty comes in recognising and treating complications.

Treatment of complications revolves around prevention and treatment of apnoea. Mitchell and Burton (1991) stated that the apnoea during anaesthesia of southern elephant seals does not necessarily indicate a problem as many elephant seals have undergone prolonged apnoea during anaesthesia without apparent ill effects. Results of this study indicate that the animals do appear to have an amazing ability to survive prolonged apnoea and can, with the use of mechanical ventilation, recover from what appear to be severely anoxic states. However, the pharmacokinetic and blood gas data

indicate that these parameters are affected during apnoea and, because it can be difficult to predict which animals will survive, apnoea during anaesthesia should be avoided.

The data also indicate that apnoea should be expected during restraint (Chapter 13). However, in most cases it can be prevented or treated using physical stimulation (Chapter 13); further indicating the need to consider any period of apnoea to be abnormal and warrant treatment. In cyanotic or deep animals the only reliable treatment for apnoea is intubation and ventilation. The ability to intubate and ventilate seals always needs to be available when restraining them and therefore techniques for ventilation of large seals need to be developed as a matter of priority. It should also be stressed that some animals can die without signs of cyanosis or central depression; it can be very difficult to tell which animals these will be even with experience and therefore when in doubt animals should be intubated and ventilated.

It is still not understood why some animals die during anaesthesia and others survive. This study has implicated a number of associated factors including presence of vomitus and instruments in the upper respiratory tract, variations in anaesthetic concentrations in the blood and changes in venous blood gas values associated with apnoea. However, pH, blood gas and anaesthetic drug concentrations measured in the extradural intravertebral vein may not accurately reflect central values and to further research the relative contribution of these factors toward death blood samples will need to be assayed from the extradural intravertebral vein at the cervical level (reflecting brain concentration) hepatic sinus (reflecting the reservoir concentration) and arterial supply to the brain whilst correlating levels with pharmacodynamic changes. The evidence does, however, suggest that death is associated with apnoea, prolonged high peripheral anaesthetic concentrations and radical changes in blood gas values in the extradural intravertebral vein. These data suggest that the theory of anaesthetic drugs pooling in the hepatic sinus during apnoea and being released into the central circulation at high concentrations, during and at the termination of apnoea, and inappropriate dive responses, and rapid development of anaerobic conditions, may all be factors contributing to death during anaesthesia (Backhouse 1964, Mitchell and Burton 1991).

The key to management of apnoea during anaesthesia may depend upon either finding the stimulus for it and blocking it, or finding the stimulus to recommence breathing and activating it. Future work should examine the control of respiration during anaesthesia in these animals and the changes in circulation during apnoea. The use of neuromuscular blocking drugs to routinely allow intubation and ventilation of all restrained animals may be preferable to allowing animals to breath spontaneously.

Control of the duration of restraint can be improved by using antagonists (Chapter 12), and breathing can be stimulated by using doxapram (Chapter 11), however neither of these treatments can be relied upon to treat apnoea. If the kinetics of these drugs are similar to those of ketamine during apnoea (concentrations being prolonged in peripheral blood at high levels), their use at these times may be

contraindicated, as it could possibly predispose to myocardial ischemia, or drug over-dose if circulation returns.

The time when invasive data-logging devices will be used to research various aspects of seal ecology is rapidly approaching. Deployment of these data-loggers may require surgical implantation necessitating deeper and more prolonged anaesthesia. Concerns about the safety of the anaesthetic episode should not preclude their use but need to be considered and the use of a dedicated anaesthetist in these studies is indicated. Gaseous anaesthesia after sedation with one of the drug combinations mentioned in this study may improve the safety and control of the anaesthetic episode and, with planning and foresight, could be used in any but the most remote and inaccessible locations.

Finally it is hoped that this work has helped put anaesthesia of these animals into its true perspective as a potentially dangerous and life threatening procedure. It is, however, a procedure which, with experience and the ability to intubate and ventilate animals, can be performed safely and routinely in the majority of cases.

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Antarctic Animal Care and Ionising Radiation Usage Ethics Committee

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Dr Rupert Woods
School of Pharmacy
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Dear Rupe,

Summary of approval from the Ethics Committee

Please find enclosed summaries of Ethics Committee approval for the years 1990-91 and 1991-92. I have also enclosed some material relating to approval for the 1989-90 field season. It appears that the production of a summary of committee approval was not introduced until 1990-91.

I hope these papers fulfil your requirements, if not please contact me again.

With best regards.

Rupert Summerson
Secretary

25 June 1992

SUMMARY OF ETHICS APPROVAL 1990-91

Investigator	**Burton, Mr Harry**
Project No	90
Project Name	The Life of the Southern Elephant Seal in the Southern Ocean
Location	Macquarie Island
Type	Experimentation involving animals.
Activity Involved	Anaesthetisation, blood sampling and attachment of devices.
Species	Mirounga leonina 60 juveniles/adults (maximum) to have time-depth recorders fitted. 30 juveniles/adults (maximum) to have satellite transmitters attached. 90 animals in total to be anaesthetised and have blood samples taken as part of the above procedures.
Waste Disposal	Not applicable.
Field personnel	The Committee requested that field personnel involved are appropriately qualified and experienced in the techniques to be used.
Remarks/Conditions	In the event that an animal dies during an anaesthetic episode a full post-mortem must be provided for the Committee's consideration as soon as possible after the death.
Date(s) of Ethics Consideration	**27 April 1990**

SUMMARY OF ETHICS APPROVAL 1991-92

INVESTIGATOR	Burton, Mr Harry
PROJECT NO	520
PROJECT NAME	An anaesthetics assessment study of Elephant seals at Macquarie Island
LOCATION	Macquarie Island
TYPE	Experimentation involving animals.
ACTIVITY INVOLVED	Weighing, pharmokinetics, tagging
ANAESTHETIC(S)	See summary below
RADIO ISOTOPE	Not applicable.
SPECIES	Southern Elephant seal (*Mirounga leonina*)

The following is the Committee's agreed position regarding animal usage, animal numbers listed here are as requested:

Southern Elephant seal (*Mirounga leonina*)
Total to be affected is 152 Adults and 600 pups and/or weaners..

Adults (males)
Pharmokinetics: 10
Tagged: 10

Adults (females)
Premoult adults: 110
Pharmokinetics: 110
Tagged: 110

Post partum to be affected: 12 (See remarks below)
Pharmokinetics: 12.
Tagged: (same) 12.

Late lactating to be affected: 20. (See remarks below)
Pharmokinetics: 20.
Tagged: (same) 20.

Pups (new born and weaners)
Total to be affected: 600. (See remarks below)
Weighing: 600.
Tagging: (same) 600.

Part 1. Pharmokinetic studies

A summary of animals to be anaesthetised at Macquarie Island in 1991-1992 including age, sex, physiological state and drugs to be used.

Anaesthetic combination	Dose mg/kg	No. of animals	sex	age years	physio. state
1. Zoletil/ ketamine and diazepam comparison and attempted drug reversal					
i. Ketamine HCl attempt reversal with:	4-6 combined with diazepam 0.1.				
a. 4-AP	0.3	20	f	4-5	premoult
b. Ro15-1788	5	20	f	4-5	premoult

ii. Zoletil 0.8-1.0
attempt reversal with:

a. Ro15-1788	5	20	f	4-5	premoult
b.Normal saline/control		20	f	4-5	premoult

iii. Zoletil 0.8-1.0 increase sample size

		8	f	4-5	premoult

2. Pethedine reversal trial
Midazolam 15 mg total dose combined with pethidine 1.5 g total dose, give thiopentone sodium 5% IV to effect then:

a. Diprenorphine	5mltotal dose	5	f	4-5	premoult
b.Normal saline/control		5	f	4-5	premoult

3. Adjusting the ratio of ketamine to diazepam
Administer 1.2 g ketamine and 30 mg diazepam

a.		12	f	4-5	premoult

4. Positioning trial

Ketamine HCl	4-6 combined with				
diazepam	0.1	10	m	6-9	premoult

5. Completion of pharmacokinetics trials begun in 1990

Ketamine HCl	4-6 combined with				
diazepam	0.1	12	f	4-5	post partum
		20	f	4-5	late lactation

Total animals to be anaesthetised: 152

Note: all animals will receive 0.005 mg/ kg atropine sulphate combined with these drugs as a parasympatholytic.

Part 2. Studies in temporal and and geographic changes in maternal investment by weighing pups at birth and at weaning.

Summary of young animals to be handled:

Pups (new born and weaners)

Total to be affected: 600. (See remarks below)
Weighing: 600.
Tagging: (same) 600.

Field personnel

Dr R Woods, Ms Lynne Robinson, Mr Peter Lynch

AREG advice

1. *Anaesthetics* The meeting considered the anaesthetics component of the project to be well thought out and noted that the questions raised by the Ethics Committee regarding drug dosages etc need to be resolved. It supported the completion of this component of the proposal.

2. *Pupil Dilation* The sub-committee agreed with the Ethics Committee that the objectives for the pupil dilation component are not clearly identified. It was considered that there is some scientific merit in undertaking this study but recommended that the study be undertaken on weaners as these animals are less important in the population structure and are sedentary at this stage, and will therefore be easy to study. Care should be taken to protect the animals from excessive ultra-violet light. One suggestion was that the animals should be enclosed in a covered cage. The sub-committee recommended that a maximum of five animals should be studied.

3. *Tagging/Weighing* The meeting endorsed the Ethics Committee recommendation that the cold-branding technique be evaluated. AREG advised that this technique is not suitable for new born pups, and recommended that they should be tagged. The sub-committee endorsed the questions raised by Ethics and supported the suggestion that they be passed back to the proponent for clarification.

Remarks/conditions

Part 1. The Committee feels that the project has made great progress in an important direction and wishes to encourage further research. The use of premoult adults is approved. The committee does now approve the use of lactating/post-partum females.

The view of the Committee is that abandoned pups shoulld be subjected to euthanasia unless in the view of the vet or responsible person that the pup has a high chance of survival. Should an abandonment occur the researcher should inform the committee without delay.

Part 2. Approved.

The Committee did not approve the pupil dilution component of this project. A letter setting out the committee's reasons will be forwarded to the Cheif Investigator.

Date of Ethics Consideration

1 August 1991

It has been conventional in veterinary medicine to express the doses of drugs as quantities per unit of body weight. This principle works well if applied to individuals of approximately the same size and usually of the same species. However, if the dose is stated as quantity of drug to be given per kcal per day (MEC dose), then it can be used for all species of all sizes that absorb, metabolise and distribute the drug in the same way (Gibbons et al. 1988). Table Appendix II presents drug dosages used during this study in mg/kg and as MEC dose.

Appendix II. Drug dose rates (mg/kg and mg/kcal/day; minimum energy cost (MEC) dose) determined using lean or total body mass and based on doses found to be useful in a model 400 kg pre-moult female southern elephant seal of estimated MEC = 6261 kcal/day. (MEC (kcal/day) = K (mass[kg]^0.75) where K is the energy group for placental mammals = 70 kcal/kg (Gibbons et al. 1988, Sedgwick and Pokras 1988)). Lean body mass for animals of other categories or at other times of their yearly life cycle can be estimated using the relationships presented in Appendix V.

Drug combination	Route	Model dose (mg/kg) Based on		MEC dose (mg/kcal/day) Based on	
		Total body mass	Lean body mass*	Total body mass	Lean body mass*
100:1 ketamine:	i.m.	3.00		0.19	
diazepam		0.03		0.0019	
50:1 ketamine:	i.m.	3.00		0.19	
diazepam		0.06		0.0038	
Ketamine and	i.m.	3.00		0.19	
xylazine		0.50		0.032	
Ketamine and	i.m.	3.00		0.19	
midazolam		0.02		0.0013	
Tiletamine and	i.m.	0.50		0.032	
zolazepam		0.50		0.032	
Midazolam,	i.m.	0.04		0.0026	
pethidine and		4.00		0.26	
thiopentone	i.v.	3.00	4.55	0.19	0.17
Midazolam,	i.m.	0.04		0.0026	
pethidine and		4.00		0.26	
ketamine	i.v.	3.00	4.55	0.19	0.17
Doxapram	i.v.	2.00		0.13	
Yohimbine	i.v.	0.06		0.0038	
Naloxone	i.v.	0.01		0.0064	
Additional dosages required to induce level 3-4 restraint in animals at level 1 or 2 restraint					
For 100:1 ketamine: diazepam					
Ketamine	i.m	1.50		0.096	
Ketamine	i.v.	1.00	1.52	0.064	0.058

*Lean body mass = total body mass - (34{total body mass}/100) (M. Hindell unpublished data).

Appendix III: Contents of emergency kit. These items were carried in a small back pack whenever an animal was to be chemically restrained (Plate 3.5). All attachments and fittings should be checked prior to use to ensure that they are the correct size and work. (OD = outside diameter).

Item		Description	Comment
Container		45 x 20 x 10 cm metal tool box	Strong (so as to resist trampling by bulls), light, transportable and easy to open and close.
Assorted syringes and needles	Syringes	2 x 50 mL	Useful for doxapram
		2 x 20 mL	
		2 x 10 mL	
	Needles	10 x 18 G x 38 mm	
		3 x 18 G x 90 mm	
Equipment for intubation	Tapes	2 x 100 cm	To hold jaws open (38 mm climbing tape is useful). 1 for top jaw, 1 for bottom jaw. Knots, or loops tied in end give better purchase.
	Endotracheal tubes	26 mm OD	Cows/bulls
		12 mm OD	Yearlings/ laryngeal spasm
		9 mm OD	Yearlings/ laryngeal spasm (kept in rectal gloves)
	Nasal oxygen tubes	2 x 20 cm soft plastic about 15 mm OD	
	Obstetrical lubricant	50 mL	
	Artery forceps		To clamp cuff of endotracheal tube though usually not necessary.
Equipment for ventilation	Demand valve and connector		Hudson 5090 is ideal for cows
	Regulators	C size	To fit C size O2 cylinder
		D/E/G size	To fit D, E, and G size O2 cylinder
	C size oxygen cylinder	Medical grade	With knob to open it
Drugs	Doxapram 5 x 20 mL	5 x 20 mL	
	Diazepam 10 x 2 mL	10 x 2 mL	
	Adrenaline tartrate injection	1 x 5 mL	
	Pentobarbitone sodium	1 x 100 mL	
	Suxamethonium chloride	3 x 2 mL	
Other	Duodenal tube		With end cut off or modified for luer fitting; for introduction of doxapram.
	Oesophageal stethoscope		

Appendix IV: Fatalities

There were two fatalities during this study (Chapter 13). A brief summary of each anaesthetic episode and discussion are presented below.

Animal 3071 (Figure 13.1D)

Anaesthetic episode

Drug doses: ketamine = 3.96 mg/kg, diazepam = 0.04 mg/kg. This animal became apnoeic at 13 min (level 6 restraint) when gastric lavage was performed. Heart rate was relatively high, 103 bpm at 18 min. The animal was intubated at 21 min during which, and immediately after, there was arching of the neck and opening of the jaws which clinically resembled the physical process of vomiting, though no vomitus could be seen in the buccal cavity. Two min later heart rate had dropped to 64 bpm. Palpebral response remained brisk. The animal was able to respond to external stimuli and was considered to be at level 5 restraint. There was frequent quivering of the external nares and facial musculature. At 42 min heart rate had dropped further to 16 bpm. The animal was given 4000 mmol bicarbonate intravenously as a bolus into the extradural intravertebral vein, then an infusion of Hartman's solution was administered and positive pressure ventilation commenced at a rate of 12/ min. At this stage the animal appeared lightly immobilized (level 4) and responded to vocalisations made by other seals around her. The palpebral response was brisk and there was constant twitching of the external nares and facial musculature. There was a rapid increase in pH and HCO_3^-. Pv_{O2} increased over the next 15 min then remained relatively stable (approximately 15 - 20 mmHg) except for falls at 82 min (immediately after 1000 mg doxapram had been administered) and at 145 min when the frequency of ventilation was decreased to allow CO_2 to build up. Heart rate fell to 3 bpm or was undetectable. Mental state as judged by palpabral response and response to external stimuli continued to decline. At 155 min 0.2 mg isoprenaline was given intralingually in an attempt to increase strength and rate of contraction of the heart. There was no observable effect. The animal's mental state continued to decline until at 180 min responses were hard to elicit, mucous membranes were muddy and cyanotic and a heart rate could only be detected with difficulty. At 190 min 30 g of pentobarbital sodium was administered into the extradural intravertebral vein. The animal died 3 min later. Packed cell volume rose from 64% at 10 min to 69% just prior to death. On post mortem vomit was found in the oro and nasopharynx but not in the mouth or trachea. Blood was pooled in the abdominal sinuses.

Discussion

It was puzzling at the time why this animal would not commence spontaneous respiration. It appeared to be only lightly anaesthetised, the airway was being held open by the endotracheal tube and there appeared at least initially to be minimal central nervous system depression. There was response

to vocalisations of animals around her. This, coupled with the twitching of her external nares and sporadic relaxing and contracting of the facial musculature indicated an animal with both a desire and the ability to breath. Ketamine concentrations, at least initially were not excessively high (about 3000 ng/mL, similar to initial concentrations seen in animals breathing normally), and dropping to levels which did not prevent restraint in other seals (< 1500 ng/mL) indicating that some factor other than ketamine concentration was responsible for preventing breathing. In this animal apnoea was induced by gastric lavage and may have been maintained by passage of the endotracheal tube (shown to prevent breathing in light animals Chapter 11). The animal appeared to vomit on intubation though no vomitus was evident in the buccal cavity on examination. It was thus interesting that vomitus was found within the buccal cavity and upper oesophagus on post-mortem. This fluid could not be seen per os as the caudal margin of the soft palate and the base of the tongue make a seal between the buccal cavity and the entrance to the larynx. The entrance to the larynx appears to open directly into the nasal choanae forming an airway that can effectively be shut off form the buccal cavity. This observation is important as it shows that because of the anatomy of the upper respiratory tract, vomited or regurgitated material may not be seen on examination per os despite its presence. Therefore if an anaesthetised animal appears to vomit but no vomitus can be seen per os the possibility of vomitus being present in the nasopharynx should be considered and the animal intubated to protect the airway until the swallowing reflex is regained. It shows the importance of an endotracheal tube in animals which vomit, the tube allowing the airway to be maintained and lungs protection from aspiration of stomach contents, bronchospasm and aspiration pneumonia. This may be why this animal did not breath.

The post-mortem findings in this animal which were thought to indicate that death was associated with a "dive-reponse" are questionable. Over a five year period we have post-mortemed many southern elephant seals which have been found dead from causes unrelated to anaesthesia on the beaches at Macquarie Island. In all cases the same evidence of circulatory changes ie pooling of blood in the abdominal and thoracic sinuses were seen. This leads us to suspect that this is nonspecific agonal change and to question what in fact a "dive-response" really is. In our opinion it appears more likely to be a consequence of drug over-dose or other factors induced by anaesthesia such as vomiting, rather than a normal part of seal anaesthesia.

It is likely that the vomitus in the nasopharynx, intracellular brain acidosis induced by administration of bicarbonate and the failure to intubate and mechanically ventilate the animal before it became anoxic contributed to its death. The only effective treatment for respiratory acidosis in these animals is intubation and ventilation (preferably with O_2) to remove the cause: CO_2. This animal could have been saved if the upper airway had been checked, intubation postponed and bicarbonate and other drugs not administered.

Animal 3049 (Figure 13.1E)

Anaesthetic episode

Drug doses: ketamine = 3.47 mg/kg, xylazine = 0.36 mg/kg. This animal became apnoeic 4 min after the drugs had been administered. Gastric lavage was performed at 10 min, the animal weighed and yohimbine (approximately 0.1 mg/kg) and 4-aminopyridine (approximately 0.3 mg/kg) administered into the extradural intravertebral vein at 25 min. A bicarbonate and saline infusion (1000mmol/L HCO_3^-) was commenced at 27 min and several deep breaths taken at 33 min. At 35 min 1000 mg doxapram was administered into the extradural intravertebral vein, breathing stopped and at 37 min violent muscular fasciculations and extensor muscle rigidity commenced. The animal was unapproachable during this period. At 56 min shaking stopped and the pupils began to dilate. The animal lost all muscle tone and a heart rate could not be detected. The relaxed musculature allowed immediate intubation and the animal was ventilated with oxygen. Pupils returned to their normal miotic state by 58 min. Breathing commenced at 62 min and assisted ventilation continued with each breath until 80 min when respiratory pattern became erratic and it was decided to further assist respiration. Consequently mechanical ventilation was recommenced. Shortly after this time there was pupillary dilation, loss of all muscle tone and a heart rate could not be detected. Positive pressure ventilation continued for a further 5 min when at 85 min the animal was declared clinically dead. Packed cell volume rose from 69% at 5 min to 80% just before death. There was pooling of blood in the abdominal sinuses on post-mortem.

Discussion

This animal could not be intubated until 56 min because it would have bitten the operator. Use of skeletal muscle relaxants enabling safe access to the larynx to allow intubation of lightly anaesthetised animals might have helped (see Ling *et al.* 1967). (We have found that if diazepam or suxamethonium are administered within min of the onset of apnoea they will usually have an effect and allow intubation in anoxic, light cows; presumably circulation to the brain and tissues is still maintained and takes time before shutting down?)

The effect of the yohimbine, 4-aminopyridine and doxapram was distressing. The animal went into opisthotonus, the front flippers were held raised, supine in extensor rigidity and the whole body shook violently. It is likely that their administration compounded the problem of anoxia and use of antagonists and central nervous system stimulants may be contraindicated in apnoeic animals.

Gastric lavage, use of antagonists and the inability to intubate and ventilate the animal before it exceeded its aerobic dive limit may have contributed to its death. This animal could have been saved if it had immediately been intubated and ventilated with oxygen.

Appendix V: Lean body mass

Lean body mass has been calculated for male southern elephant seals > 510 kg using the relationship:

Lean body mass = total body mass - total body fat (Slip *et al.* 1992)

Where:

Total body fat = {[1.178][total blubber mass]} + 0.591

and

Total blubber mass = -574.518 + {[225.802][C_3)} + {[85.628][snout-tail length]}

(C_3 = maximum girth, C_3 and snout-tail length in m, body mass in kg).

There is little information on body composition in female and male seals < 510 kg. Based on body composition data determined in post-partum and post-breeding females respectively, M. A. Hindell (unpublished data 1987) considered total body fat to be 34% of total body mass in pre-fast and 27% in post-fast mature females. These figures could be used for males < 510 kg until further data become available.

Appendix VI: Complications during chemical restraint

This appendix presents the anaesthetic related complications most commonly encountered during this study, and the techniques which were used to recognise, prevent, and treat them.

Recognition of problems

Expected normal responses, and those considered abnormal, when restraining animals to level 3 or 4 restraint have been presented in Table 6.3.

General guidelines to prevent complications

Most complications during southern elephant seal anaesthesia can be avoided or minimised by observing the following general guide-lines.

- Use the lowest dose of drug, and work at the lowest level of chemical restraint appropriate for the procedure being performed.
- Maintain restraint for the minimum possible time.
- Monitor the anaesthetic closely.
- Select quiet, somnolent animals, away from water or wallows.
- Position animals in a horizontal plane, with the neck extended. Consider positioning larger (≥ 1000kg) animals in oblique recumbency.

General guidelines to facilitate treatment of complications

- Ensure that there is easy, safe access to the animal.
- Be able to recognise problems, know how to treat them, and have equipment available to do so.
- Place an intravenous catheter immediately it is safe to do so.

Anaesthetic related complications

Inappropriate anaesthetic depth

Levels of chemical restraint required for various procedures in southern elephant seals are: venipuncture = level 3 - 4, intubation (small seals (< 1000 kg) = level 5, large seals (≥ 1000 kg) = level 6), weighing (small seals = level 4-5, large seals = level 6), gastric lavage (small seals = level 4 - 5, large seals = level 5 - 6), minor surgery (level 4 - 5, when combined with local anaesthesia or level 6 alone).

Problems of anaesthetic depth fall into three categories: (1) inadequate depth, (2) excessive depth and, (3) prolonged anaesthesia or recovery.

Appropriate anaesthetic depth can be achieved by administration of appropriate drug doses. If mass is not known, an estimate can be made from length and girth measurements (Chapter 5), or nominal total dose rates (Chapter 6), or MEC-dosages (Appendix II) can be used.

Excessive levels of chemical restraint can be treated by administration of analeptics or antagonists to decrease or terminate restraint in animals which are breathing (dosages of these drugs are presented in Table VI.1), and / or the use of assisted or positive pressure ventilation (Chapter 3). Administration of antagonists and analeptics to apnoeic animals may exacerbate anoxia and until more information becomes available, should be avoided if possible.

Ventilatory problems

The three most common ventilatory problems in southern elephant seals are: (1) apnoea, (2) slow, shallow or stertorous breathing and, (3) laryngeal spasm.

Treatment of apnoea has been discussed (Chapter 13, see Figure 13.2). The most important indicators for intubation and ventilation with oxygen during apnoea are cyanosis and deep anaesthesia. Intubation and positive pressure ventilation (Chapter 3 and 13) is the treatment of choice. Appropriate endotracheal tube sizes are presented in Table VI.2.

Laryngeal spasm is usually associated with an acute onset of whole body heaving, gagging, and/ or stridor which may last up to 2 min. There are multiple attempts at inspiration with simultaneous whole body heaving* and stridor can progress to apnoea. Animals rapidly become cyanotic and death can follow. It is uncommon, occurring in < 1% of all anaesthetic episodes. Vomiting can also cause these signs so it is important to differentiate between the two problems by inserting the hand into the oropharynx and feeling for vomit. A flow chart for treatment of breathing difficulties has been presented in Chapter 13, Figure 13.3.

Poor cardiac function

The most common circulatory problems during anaesthesia are cyanosis, bradycardia and tachycardia associated with apnoea which is best prevented or treated.

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* These signs can be seen in animals to which drugs have not been administered though the incidence appears few; we have seen them in 2 cases over 4 field seasons work with these animals. This suggests that these signs are anaesthetic related.

Table VI.1. Drug dosages used in treatment of complications associated with chemical restraint in southern elephant seals. The incremental dose of tilatamine and zolazepam is the dose of each constituent.

Drug	Dose (mg/kg)	Comment
Diazepam	0.04 - 0.06	Total dose for a cow is 15 - 25 mg, to effect.
Doxapram	2.00	Transient effect in breathing animals so may need to be repeated. Avoid in apnoeic animals, however if positive pressure ventilation is not available it can be administer endotracheally.
Midazolam	0.1 - 0.2	Total dose to sedate a 50 kg pup is 5 - 10 mg i.m (n = 5; maximum sedation in 10 - 15 minutes). Caution if administered intravenously as it can cause apnoea (n = 2).
Naloxone	0.01	Give intravenously. Use a concentrated preparation.
Pethidine	2.0 - 4.0	Total dose to sedate an anxious cow (level 2 - 3), previously restrained with 3.0 mg/kg ketamine and 0.03 mg/kg diazepam is 1 - 2 g intramuscularly (2 mg/kg). A similar dose can be used with 15 mg midazolam to calm cows used for fostering.
Suxamethonium	0.5 - 2.0	Give to effect. Onset indicated by fine muscle fasciculations, facilities for intubation and positive pressure ventilation must be available (see Ling et al. 1967)
Yohimbine	0.06	
Further anaesthetic		All are given to effect, preferably intravenously. Avoid rapid intravenous administration.
Ketamine	1.00	30% of initial dose.
Thiopentone	1.0 - 3.0	When combined with midazolam and pethidine.
Tiletamine and zolazepam	0.025 - 0.100	5 - 20% of initial dose intravenously.

Table VI.2. Lengths, masses, and endotracheal tube sizes for different classes of southern elephant seal at different times of their life cycle. Endotracheal tubes are usually left uninflated, however they can be inflated if necessary. Categories of animals are described in Chapter 6.

Category			Snout-tail length (m)	Mass (kg)	Endotracheal tube size (outside diameter) (mm)
Sex	Age	Stage of yearly cycle			
Male and female	Yearling		1.88 (1.63-2.30)	190 (119-417)	10.0, 12.3 or 15.7
Female		Prefast	2.55 (2.10-2.95)	469 (283-700)	24.0*
		Postfast		366 (278-576)	
Male	Subadult				24.0 or 30.0
	Bachelor	Prefast	3.82 (3.43-4.05)	1855 (1806-2038)	
		Postfast		1334 (1031-1567)	
	Harem bulls				
	I. Challenger	Prefast	4.00 (3.7-4.24)	2486 (1574-2635)	30.0 or 35.0
		Postfast		1518 (1334-1758)	
	II. Beach master	Prefast	4.33 (4.0-4.81)	2693 (2068-2897)	
		Postfast		1854 (1666-2416)	

*Endotracheal tube size is similar for similar sized males. When a larger tube can not be passed due to tight arytenoid adduction, 15.7 or 20.0 mm outside diameter tubes can be used.

Detection of decreased ventilation, abnormal breathing pattern or apnoea, soft, slow (< 10 bpm), irregular or absent heart sounds, prolonged capillary refill time, cyanosis, dilated pupils, and depression or loss of consciousness are important indicators of impending arrest or poor cardiac function. During cardiopulmonary arrest there is profound muscle relaxation, no ventilation, and no detectable heart beat. Pupils become dilated and nonresponsive to light. Pupillary dilation is a very poor prognostic sign, however in most cases this can be reversed by ventilating the animal, observing the pupil size and mucous membrane colour to determine the effectiveness of ventilation. Positive signs include return of detectable heart beat, improved membrane perfusion and colour, and pupillary constriction.

Hyperthermia

Hyperthermia occurs rarely, in an estimated <1% of all immobilizations. Cooling should occur if hyperthermia is suspected (using water and supplying shade; rectal enemas of cold water could also be considered). If rectal temperatures cannot be taken then cooling should be instigated immediately it is safe to do so when working at temperatures ≥ 5°C or on sunny days. Shaking should be minimised.

Vomiting, regurgitation and aspiration of stomach contents

Vomiting is usually preceded by any or all of the following; gagging, heaving body movements, an arched neck, opening of the mouth, apnoea, stertorous breathing, and vomitus may spill out of the mouth or nose. Vomiting is seen in <1% of all animals immobilized unless they are intubated or a gastric lavage tube passed. In these cases the frequency increased to < 10 % of these animals.

Vomiting can be difficult to recognise as in some cases vomit will be trapped in the nasopharynx, will not flow from the nose, and cannot be visualised by mouth. If vomiting is suspected, and no vomitus is apparent, it is thus important to insert a hand into the mouth, through the sphincter made by the base of the tongue, the soft palate and aryepiglotic folds and to feel for vomitus to enable a differentiation to be made between vomiting and laryngeal spasm, the presenting signs of which can be similar. Vomitus is usually fluid containing squid beaks and parasites and thus not usually made up of food. It will feel hot when a cold hand is introduced into the oropharynx.

Vomiting tends to occur at light levels of chemical restraint (≤ level 5), and is most commonly associated with intubation or passage of a gastric lavage tube. Regurgitation is also seen but is usually associated with deeper levels of chemical restraint (> level 5), vomitus is usually clearly visible, and there are usually no signs of body heaving or gagging.

Minimising the incidence of vomiting is difficult because it is safest to work with animals at low levels of restraint. Starving of animals prior to chemical restraint may decrease its incidence, however we have seen vomiting or regurgitation of fluid in post-moult females which had been starving whilst ashore for up to 3 weeks. Premedication may also be useful however this can be difficult under field

conditions. Use of xylazine has also been implicated in deaths due to aspiration of stomach contents (Mitchell and Burton 1991) and this drug could be avoided.

To decrease the chances of regurgitation during gastric lavage, use of excessive volumes of water at any one time should be avoided as this fluid can reflux back around the tube (Chapter 13). The chances of aspiration pneumonia occuring can be decreased by positioning the patient with the head slightly down hill or by endotracheal intubation prior to gastric lavage. Regurgitation can also be minimised by avoiding deep levels of anaesthesia.

Evidence suggests that animals will not breath if vomitus impinges upon the opening of the larynx. Remove vomit form the mouth and treat for apnoea. If possible the animal can be placed slightly head down to aid drainage of vomitus.

Shaking

Shaking requiring treatment occurs in < 1% of all immobilizations and is seen under three circumstances during chemical restraint of southern elephant seals: (1) The most common occurrence is during routine chemical restraint when using cyclohexamine based drug combinations. Shaking is usually mild (fine muscle fasciculations; level 1) and resolves spontaneously; (2) Less commonly level 1 shaking may progress to become coarse (level 2), which may or may not be followed by whole body spasming (level 3) and/ or status epilepticus (level 4); (3) Shaking can be seen after anaesthetic antagonism or use of respiratory stimulants in breathing animals (usually level 1) or within minutes of return of breathing in apnoeic animals previously treated with these drugs.

If cyclohexamine drugs are used they should be combined with an appropriate dose of sedative to minimise shaking. The incidence of shaking appears to be lower if ketamine and xylazine, tiletamine and zolazepam, or pethidine based drug combinations are administered. To prevent progression of level 1 shaking, diazepam can be administered at the first sign of level 2 shaking. Shaking can be minimised after administration of antagonists or respiratory stimulants by using appropriate doses of drugs and by administering antagonists after the anaesthetic has mostly worn off. Partial antagonism, or repeat administration of low doses of drugs may also be more appropriate than complete antagonism using high doses of drugs.

Prevention of shaking after return to breathing in apnoeic animals pre-treated with antagonists or respiratory stimulants can be difficult. Avoidance of apnoea and use of intubation and positive pressure ventilation rather than chemical techniques for stimulating breathing in apnoeic animals may lessen this problem.

A flow chart for treatment of shaking in mature female southern elephant seals is presented in Figure VI.1. Caution should be exercised in administering diazepam to apnoeic, deeply anaesthetised shaking

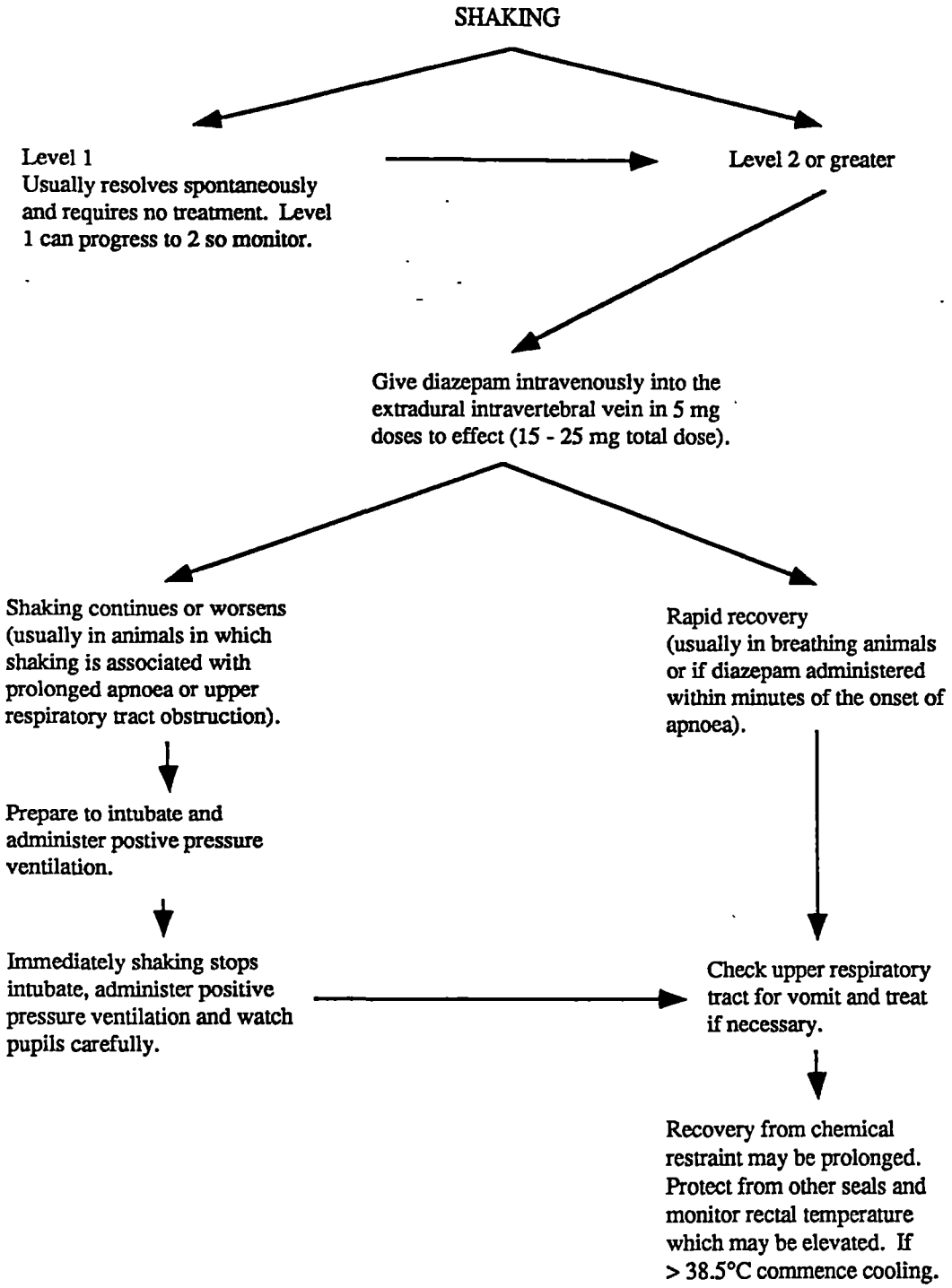

Figure VI.1. A flow chart for treatment of shaking in mature female southern elephant seals. (Shaking: Level 1 = slight/ fine, 2 = Coarse, 3 = Spasms (clonic-tonic), 4 = Status epilepticus).

animals as it can further depress the animal and lead to prolonged recovery. In these cases positive pressure ventilation or assisted ventilation may be required immediately breathing returns.

It is important to distinguish between drug induced shaking and the body heaving which may be associated with vomiting or upper respiratory tract obstruction obstruction as treatments differ. The heaving associated with vomiting or upper respiratory tract obstruction obstruction has an acute onset, is usually associated with the commencement of apnoea not its termination, and there may be gagging, stertorous breathing, an arched neck, and/ or the presence of vomitus in the upper respiratory tract.

Desertion of pups

During desertion either the mother can leave the pup (which usually occurs after recovery), or the pup can leave the mother (which usually occurs during the anaesthetic episode), the latter occurrence is far more common. It is normal for the pup to wander away from the mother. The pup usually wakes up to suckle and when it cannot find a teat, or the mother does not respond to its vocalisations it will move away, often with great determination, into the harem.

There are several techniques which can be used to minimise pup abandonment: (1) don't wake the pup up, (2) sedate the pup (Table VI.1), (3) roll the female on her side so that the pup has access to a teat and place it near the teat, (4) work with sedated cows only who can still interact with the pup, (5) place the pup at the cows head end so that they can interact, (6) physically restrain the pup (this can be difficult for prolonged procedures with large pups if holding the tail or using the dog house position and can be stressful for the pup, a cage is therefore preferred), (7) keep the cow light and ensure recovery is rapid, (8) avoid the use of large doses of antagonists to speed recovery in cows as this may excite the animal, (9) stop other cows biting the pup.

Pups should be handled as gently as possible and one person designated to be responsible for the pup (it is remarkable how rapidly and how far a pup can move away searching for its mother).

Little can be done to prevent the mother abandoning the pup. We suspect that the chances of this happening may be increased when: (1) Young or inexperienced mothers are anaesthetised, so select older, more experienced cows if possible, (2) in small or unstable harems where cows are constantly harassed by bulls, (3) on hot days when cows may over heat and try to go to sea to cool down, (4) if people are present when they wake up which might frighten them, and (5) with the use of some drugs such as ketamine and xylazine which appear to affect the animal's psyche. Cows are also more likely to leave their pups towards the end of lactation however this is usually less of a problem for the pup which has larger stores of fat than if deserted early in lactation.

If desertion occurs the pup may be cross fostered to cows known to have lost their pup recently. These cows may remain next to the carcass of their dead pup for one or two days and may accept an orphaned pup. The chances of acceptance may improve the younger the dead pup is, the younger the mother, the more rapidly the two can be put together and if the skin of the dead pup is tied on to the foster pup. Caution should be exercised during attempted cross fostering as the mother may attack the new pup. In these cases sedation of the mother with pethidine (2 mg/kg) or midazolam and pethidine (15 mg midazolam combined with 2 mg/kg pethidine) may be useful. The pup may also try to find its deserted mother and not be interested in the new mother. In these cases the pup can be sedated with midazolam (Table VI.1). Whether anaesthesia increases the frequency of pup abandonment is unknown.

Going to sea and drowning

Animals going to sea should be expected when animals are excited or anxious and are given drugs close to the sea: the closer to the sea the more likely the animals are to fly to it. Animals which have just hauled out appear to have more chance of going to sea than those that have been ashore and are settled, as do single animals compared with those in groups or if there is a large bull as part of the group (this seems to give the animals confidence and make them feel more secure). Cows with pups, and bulls, appear less likely to go to sea than animals recently hauled ashore. Cows in harems, anaesthetised at the end of lactation however appear more likely to wean their pups early and go to sea than cows anaesthetised at earlier times. Although this will often be prevented by challengers/ bulls, these animals can and do kill cows on their way to sea whilst attempting to mate with them. This needs to be prevented.

Somnolent animals in stable circumstances away from the sea are suitable for anaesthesia. (An advantage of the remote injection technique is that once the needle is placed the reaction of the animal can be assessed. Drug administration can be stopped in animals which appear frightened or immediately flee for the sea.) Make sure they are calm, relaxed and that stress is minimised. Baffle boards, people, a tennis ball on a stick, or waving plastic sheet can be used to try to stop animals from going to sea. However some will go to sea regardless of obstacles in their path.

Once animals which have just received drugs are at sea they should be monitored for breathing. Animals at level 2-3 restraint have a characteristic swimming pattern and breathing technique. The back is often arched out of the water, expiration occurs underwater as the head is slowly raised for inspiration. Sometimes the animal will float with the head submerged in this position, breathing in these cases can often be stimulated by tossing rocks in front of or onto the animal. The animals we have seen go to sea all survived, probably because of low levels of restraint. However if possible the animals should be removed from the water by boat or using a lasso. Swimming with the animals should be considered a last resort and is contraindicated in cold conditions or during the breeding season when there is the likelihood of attention from challenger bulls. Bulls will attempt to mate

with cows in the water during the breeding season and for this reason cows should be prevented from going to sea whilst sedated at this time.

Attack by other animals

Skuas may attempt to remove the eyes of anaesthetised seals. The retractor bulbi muscle and powerful lids prevents this in even deeply anaesthetised animals however skuas should be kept away.

The most common problem with attack by other seals is during the breeding season. Anaesthetised harem bulls will be attacked by challengers, or allow challengers into the harem, causing disruption. For this reason all challengers should be kept away from the harem if a harem bull is anaesthetised. Similarly if a challenger is anaesthetised too close to a harem it will be attacked by the harem bull and these animals should be anaesthetised away from the harem to prevent this happening.

Bulls will also attack anaesthetised females. It can be very difficult to keep a bull away from an anaesthetised female, particularly if she is in oestrus (about 18 days after birth). One technique is to go to the opposite side of the harem and try to move a challenger into the harem. The Beach master will usually leave the cow alone to chase the intruder, however this is a short term solution and may need to be repeated. In circumstances where there is fear for the anaesthetised cow's welfare and the bull cannot be kept away it can be anaesthetised, however by doing this there is the risk of harem disruption by challengers. However challengers are usually easier to keep out of a harem than to keep the harem bull away from a female. An alternative might be to place a visual barrier around the female.

Challengers will also kill females leaving harems by drowning them in the surf or suffocating them by lying on them. It is thus important to prevent females from going to sea at these times if possible until fully recovered.

Appendix VII: List of publications

Woods, R., S. McLean, S. Nicol and H. Burton. In press. A comparison of some cyclohexamine based drug combinations for chemical restraint of southern elephant seals (*Mirounga leonina*). Journal of Marine Mammal Medicine XX: XX-XX.

Woods, R., M. Hindell, D. J. Slip and T. Arnbom. Submitted. Length-mass relationships in southern elephant seals (*Mirounga leonina*). Journal of Marine Mammal Science XX: XX-XX.

Woods, R., S. McLean, S. Nicol and H. Burton. In press. Use of midazolam, pethidine, ketamine and thiopentone for chemical restraint of southern elephant seals (*Mirounga leonina*). The Veterinary Record XX: XX-XX.

Woods, R., S. McLean, S. Nicol, and H. R. Burton. Submitted. Antagonism of some cyclohexamine based drug combinations used for chemical restraint of southern elephant seals (*Mirounga leonina*). The Australian Veterinary Journal XX: XX-XX.

Woods, R., S. McLean, S. Nicol, and H. R. Burton. Submitted. Use of the respiratory stimulant doxapram in southern elephant seals (*Mirounga leonina*). The Veterinary Record XX: XX-XX.

Woods, R., S. McLean, S. Nicol, and H. R. Burton. Submitted. Use of medetomidine and ketamine in southern elephant seals (*Mirounga leonina*). Research in Veterinary Science XX: XX-XX.